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On the Nature of Cancer

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THE WAR ON CANCER

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Preface

Cancer is the most frightening disease of our time and an important cause of death in children and older adults. It is clearly of cellular origin and characterised by uncontrolled growth. Even if knowledge about cancer has increased tremendously during the last years, we still do not know what cancer is in any precise molecular terms.
This book should inform about the complexity of the phenomenon of cancer, and also about interests and state of knowledge in various disciplines of Swiss Oncological Research, without any claim to reflect its complete spectrum. It presents the contributions to a Symposium on the Nature of Cancer held on the occasion of the annual meeting of the Swiss Academy of Natural Sciences in Basel on October 7th, 1982. In order to make it available to a larger public, it is published in English.

In the preparation of this volume special thanks go to the contributing authors for their high quality reports. We are also grateful for the editorial assistance by the general secretariat of the Swiss Academy of Natural Sciences and by the Birkhäuser Verlag Basel. Sincere thanks go to the president of the meeting, Professor Iris Zschokke-Gränacher, and her organising committee, who did so much to make the meeting a success.

January 1983

Hansjakob Müller
President of the Swiss Society
of Genetics

Invasive potential and actual invasion: An essay on tumor progression

Peter Sträuli

1. Invasion as a criterion of malignancy

Tumors have various means to endanger and eventually to kill their hosts. Growth of a neoplastic cell population, even if it is strictly expansive and does not encroach on neighboring normal structures, is perilous in an organ with a limited range of adaptation to increasing pressure, e.g. in the brain. Furthermore, tumors producing excessive amounts of hormones or similar agents can imperil the host irrespective of their growth behavior. Within the vast spectrum of death by neoplasia, however, these are relatively rare instances compared to the effects of invasion and their eventual sequel, metastasis. Invasion or local spread is therefore the chief characteristic of malignancy, and as long as morphology is responsible for the definitive diagnosis of cancer, the search for invasion in histological preparations remains irreplaceable.

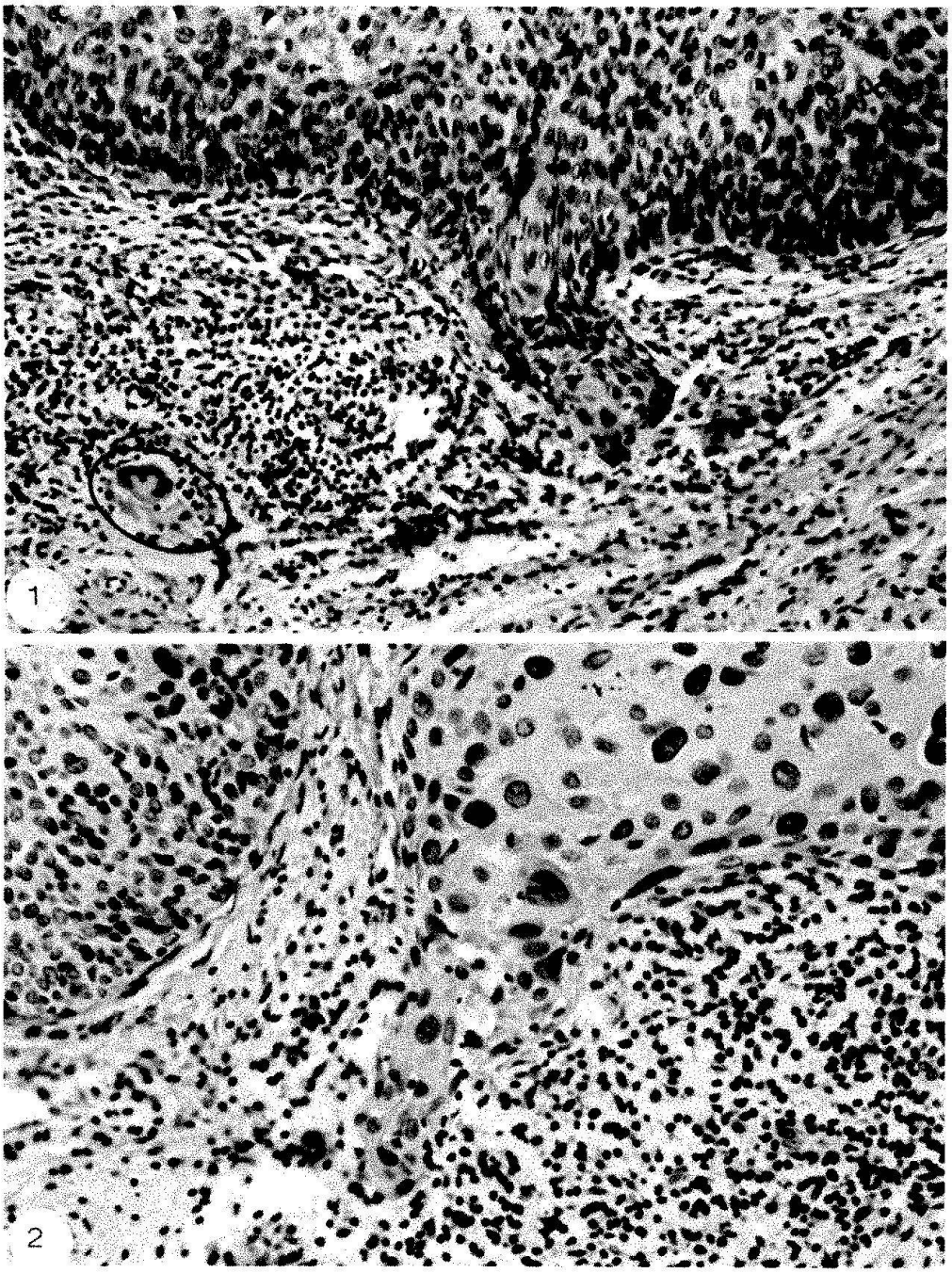
2. Principal mechanisms of invasion

It is a truism that tumor invasion is a complex process which represents, at every moment, the result of tumor action and host reaction. In spite of this complexity a rough concept of invasion, at least of heuristic value, is possible (Sträuli 1980). It must be based on those properties and activities of cancer cell populations that are regularly or at least frequently recorded in surveys of clinical and experimental malignancies. Such features are tumor cell multiplication, exertion of lytic effects, and tumor cell locomotion. Of these, proliferation is the basic property of neoplasia. Benign tumors as well as incipient stages and many metastases of malignant tumors show that cell multiplication alone is unable to achieve invasion and therefore needs support by other mechanisms. Admittedly, progressive multiplication of cancer cells (Willis 1973) is an elementary factor, even the driving force, of local spread in many types of cancer, and this is easily understandable, since two (or more) daughter cells require more space than one cell. Such space must be taken from the host, and this calls for lytic action. Thus, proliferation and destructive influences together can accomplish invasion and must not be supplemented by cancer cell locomotion. But when and where the latter becomes operative, it can increase the spead of local spread and expand its range.

To what extent are the three mechanisms of invasion operative in solid cancer? In man, the most frequent type of this tumor class is squamous cell carcinoma, of which carcinoma of the ectocervix is the most investigated representative. The available information on local spread of cervix carcinoma is summarized in the next paragraph.

3. The lesson of human cervix carcinoma

Owing to early detection by colposcopy and exfoliative cytology, innumerable specimens of cervical carcinoma were and still are subjected to histological examination. A first consequence of this endeavour was the description of a preinvasive stage with a neoplastic focus confined to the epithelium and exhibiting no tendency to extend in depth. Unfortunately, a proliferation of terms complicates communication about cervical cancer. Intraepithelial neoplasia is an adequate designation for the preinvasive stage, but the term carcinoma in situ has also become established. The lesion is intraepithelial with regard to the epithelial-stromal junction represented by the basement mem-



Figs. 1 and 2. Squamous cell carcinoma of the human ectocervix. Transition from carcinoma in situ to microinvasive carcinoma.

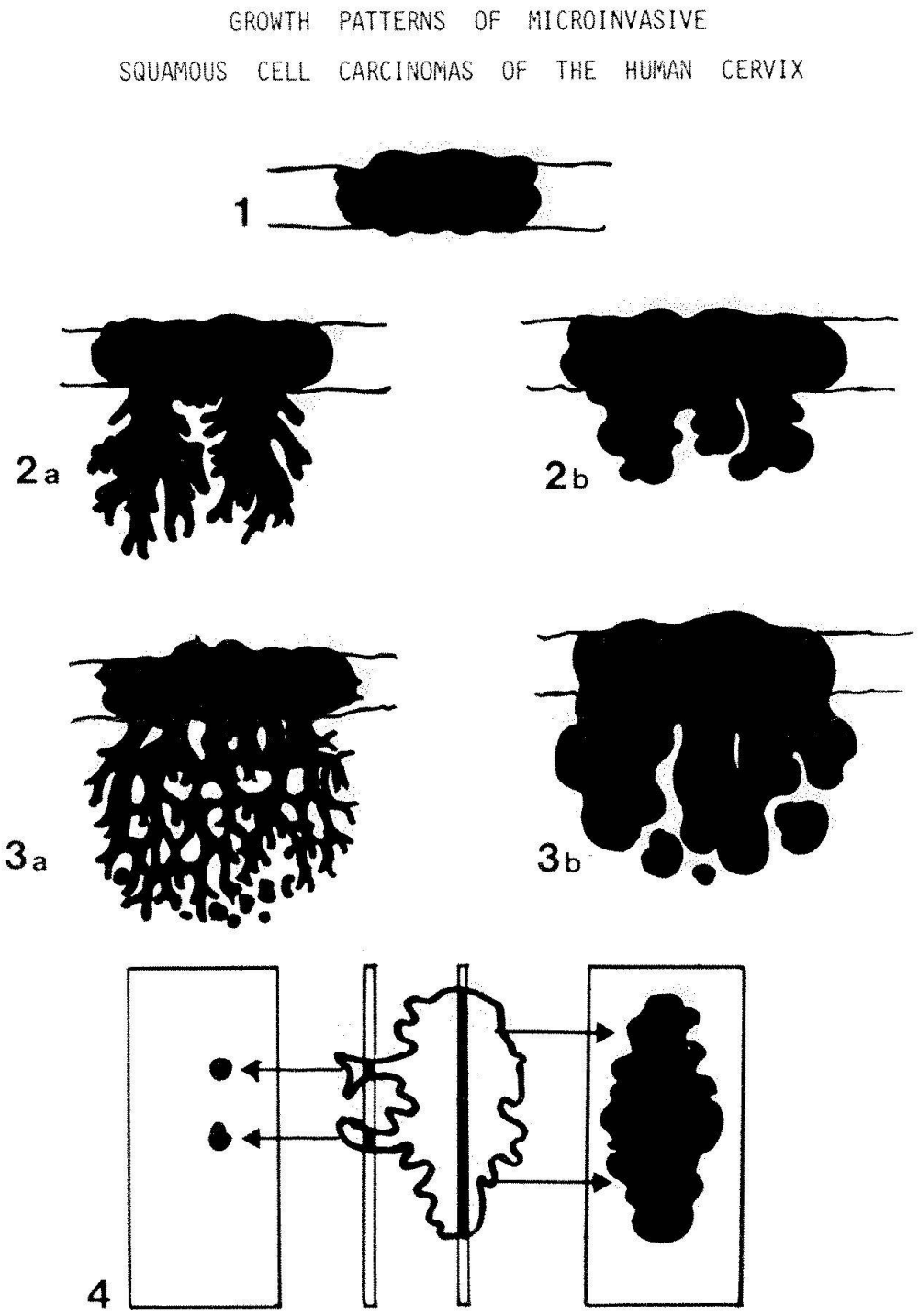
Fig. 1. A cohesive projection, consisting of pleomorphic carcinoma cells, extends from the base of the tumor into the stroma. The host tissue is infiltrated by inflammatory cells, among them a foreign body giant cell (circle). Magnification × 160.

Fig. 2. Similar situation. The pleomorphic cells in the invading part of the tumor are clearly recognizable. Magnification × 250.

Figs. 1 and 2 were kindly provided by PD Dr. C.Y. Genton, Leitender Arzt, Institut für Pathologie, Universität Zürich.

brane. However, multiplication of the neoplastic cells frequently results in their piling-up above the former epithelial surface. Many cells of these exophytically growing neoplasias are shed. This is not only the raison d'être of clinical cytology, but also a factor that slows down overall growth of the neoplastic focus.

The next stage is microinvasive carcinoma. Although there is no universal agreement on definition and description of this phase, a prevailing pattern is recognizable (Hamperl 1959, Ober et al. 1961, Burghardt 1973, Seski and Schmidt 1982). Microinvasion begins with the extension of tongues and prongs from the base of the intraepithelial tumor (fig. 1, 2). These projections consist of tightly cohesive cells which, in contrast to the undifferentiated and uniform cells of in situ carcinoma, are pleomorphic and often show features of differentiation with keratin formation. Inevitably, these tumor extensions come in conflict with the basement membrane. Recently, Spornitz and Hafez (1982) have presented excellent transmission electron microscopic evidence for the forma-



Schema 1. Growth patterns of microinvasive squamous cell carcinomas of the human cervix.
1 Intraepithelial tumor.
2 Non-confluent microinvasion: a finger-like outgrowth, b bulky outgrowth.
3 Confluent microinvasion (coalescence of individual finger-like or bulky parts): a reticular pattern, b advanced bulky outgrowth.
4 Two sections through the same tumor show (left) drop-like foci that seem to lack continuity with the tumor, and (right) the cohesive tumor mass. Serial sectioning reveals in most instances the continuity of tumor parts visible in single sections. Therefore, the distinction of a drop-like growth pattern is hardly warranted.

tion of gaps through which parts of the carcinoma cells are pushed into the stroma. Since basement membrane material is synthesized by epithelial cells, decreased supply of such material could contribute to the weakening of the basement membrane wherever the cervical epithelium is replaced by carcinoma. At the sites of actual penetration, however, proteolytic activity of the carcinoma cells must be considered as a factor of microinvasion.

In the stroma, further invasion of cervical carcinoma frequently displays two more or less successive patterns, non-confluent and confluent; in both, finer (finger-like) or coarser (bulky) outgrowths can prevail (schema 1). It must be emphasized, however, that intermediate forms do occur and that attempts to describe growth patterns should not divert from the concept of a biological and clinical entity represented by microinvasive carcinoma of the cervix.

With regard to mechanisms of invasion, two points merit special attention:
(1) Evidence for lytic effects can be found in the immediate vicinity of the microinvasive tumor parts. They consist in alterations of the stroma with degradative changes of collagen (reticulin) fibrils and the appearance of edema. Since the same sites are frequently infiltrated by host cells (lymphocytes, plasma cells, eosinophils), the origin of the lytic agents is an open issue. The tumor cells, at any rate, are serious candidates. A cathepsin B-like activity has recently been demonstrated in squamous carcinoma cells from the human ectocervix, particularly in a subpopulation with a high rate of proliferation in vitro (Pietras and Roberts 1981). It has been argued that the onset of invasion in cervix carcinoma could be due to the emergence of "a cell clone producing a proteolytic enzyme sufficient to weaken the basement membrane and the underlying collagen" (Koss 1981). Proof for this course of events appears now to be within reach.
(2) On the other hand, convincing evidence for the occurrence of tumor cell locomotion in microinvasive cervical carcinoma is lacking. Such evidence could only be of an indirect nature (direct recording of tumor cell translocation by microcinematography being excluded) and consist in the demonstration of isolated carcinoma cells or cell groups within the stroma. This can only be achieved by serial sectioning. In single sections the discontinuous localization of tumor elements is, as a rule, an artefact caused by the complex shape of the invading tumor front (see bottom of text fig. 1). So far, the strongest argument in favor of tumor cell locomotion was provided by Schiller et al. (1953), who described a few chance findings of so-called spray carcinoma. This particular type of microinvasive carcinoma is thought to develop exclusively in the basal layers of the cervical epithelium, while the higher strata remain normal; for this reason the tumor escapes detection by colposcopy or cytology. Spray carcinoma "does not show strands invading the stroma, but the invasion is performed by single carcinoma cells which form a brush- or spray-shaped structure" (Schiller et al. 1953). No spray carcinomas were found in the large material of Hamperl, Burghardt, and later investigators, and it remains doubtful whether such a particular type of infiltrating cervical carcinoma exists at all. For theoretical reasons, better evidence for tumor cell locomotion should be expected in surgical specimens from advanced cervical carcinomas; this assumption, however, is not substantiated by published reports. The conclusion is justified that invasion of squamous cell carcinoma of the cervix, at least in its early stage, is accomplished by tumor cell proliferation and by lytic events in the invasion zone, whereas a contribution of tumor cell locomotion is, at best, exceptional.

4. Tumor invasion and tumor progression

Tumor progression, the antipode of tumor regression, consists in the acquisition of higher degrees of malignancy by individual tumors. Since invasion is the main feature of malignancy, progression must be expected to be chiefly expressed in the appearence of invasiveness as such and in the emergence of the latter's more advanced stages. Tumor progression is analyzed in Fould's "Neoplastic Development" (1969), one of the master texts of theoretical oncology. A passage on carcinoma in situ of the human uterine cervix reads as follows: "To call it a cancer is dangerously misleading. Its designation as an imperfect carcinoma indicates, in harmony with the best available evidence, that the quality of invasiveness is not merely inapparent or latent but is absent and can be acquired only by progression". As pointed out in the preceding paragraph, progression from the in situ stage to the microinvasive stage implies the onset of lytic activity, but not of cell locomotion. There is some morphological evidence that this limited sequence of progression is not only characteristic for squamous cell carcinoma of the cervix, but also for other localizations of this tumor type. It is a reasonable assumption that full progression (i.e. with inclusion of tumor cell locomotion) is not or only rarely attained at the time when human squamous cell carcinomas are examined after surgery or autopsy. The same need not be true for other human cancers, e.g. malignant melanoma, although the concept of progressive invasion is basically applicable to them. On

the other hand, the limited span of progression available to squamous cell carcinomas corresponds to Fould's statement that "progression does not always reach an endpoint within the life-time of the host". The consequence of this deduction is that progression can be brought to further advance in the progeny of the original tumor through transplantation to new hosts. On this basis we can expect that an extreme degree of invasive potential can possibly be attained by transplantable squamous cell carcinomas. The crucial question, of course, is: Transplantable in what type of host? The progeny of human tumors cannot be serially transplanted in human beings. Attempts were made to use conditioned (X-irradiated and/or cortisone-treated) animals as hosts for permanently transplantable human tumors, among them several squamous cell carcinomas (Toolan 1954). These xenografts displayed capacities of invasion which, in general, did not exceed or even reach those exhibited in the patient. A noteworthy exception is H.Ep.3, the fastest growing and most invasive cancer of the human xenograft series. Its parent tumor, a squamous cell carcinoma of the buccal mucosa, was already in an advanced stage of spread when surgery was performed and material from a cervical lymph node metastasis was harvested for xenografting. In the conditioned host, H.Ep.3 displayed vigorous invasion which, judged from the published micrographs, occurred in a more dissociated growth pattern than in the original host. Spread by single cells was particularly conspicuous when H.Ep.3 was implanted on the chorioallantoic membrane of the embryonated chick egg (Gittermann and Luell 1973). As a most unusual event in this experimental approach, H.Ep.3 was found to metastasize to many organs of the chick embryo, among them gizzard, heart, lung, liver, kidney, spleen, intestine, eye, and brain. We may thus assume that this human squamous cell carcinoma reached its full invasive potential after heterotransplantation. But this is an exceptional situation. For systematic studies on dimensions of invasiveness attainable beyond the individual tumor-host association, we must resort to animal tumors. An excellent model is the V2 carcinoma of the rabbit.

5. The V2 carcinoma of the rabbit, an absolute invader

Skin papillomas of the cottontail rabbit occasionally progress to squamous cell carcinomas. From such lesions, serially transplantable tumors were established, of which V2 (or VX2) became the most widely used entity (Kidd and Rous 1940). The V2 carcinoma is a moderately to poorly differentiated squamous cell carcinoma which grows in domestic rabbits of all strains and shows a characteristic mode of spread: it penetrates into surrounding host structures from all implantation sites, produces regional lymph node metastases in most animals and spreads to the lungs in about half of the hosts.

As an example of local spread of the V2 carcinoma, some histological findings of subcutaneously implanted tumors are shown in figures 3–10. The first four photographs demonstrate characteristic growth patterns sequentially exhibited by V2 implants during two to three weeks. Cell multiplication proceeds throughout this observation period. A cohesive invasion front exists only for a short time and often abuts upon connective tissue with increased density (fig. 3). Such capsule-like structures are invaded within a

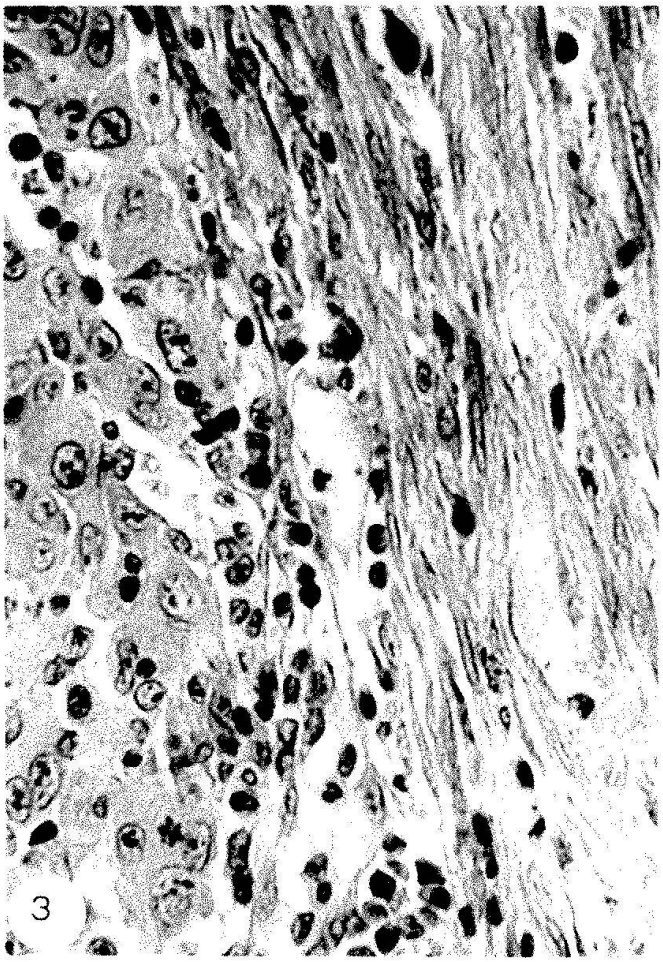
Figs. 3 - 6. V2, transplantable squamous cell carcinoma of the rabbit. Growth patterns after s.c. implantation.

Fig. 3. A cohesive part of the tumor (left) abuts on connective tissue (right) with increased density. Magnification × 500.

Fig. 4. A less cohesive part of the tumor with remnants of connective tissue. Magnification × 500.

Fig. 5. Tongue-like projections of the tumor extending into the surrounding host tissue which shows a loosened structure. Magnification × 500.

Fig. 6. A single carcinoma cell (circle) is localized at a slight distance from the invading edge of the tumor. Magnification × 500.



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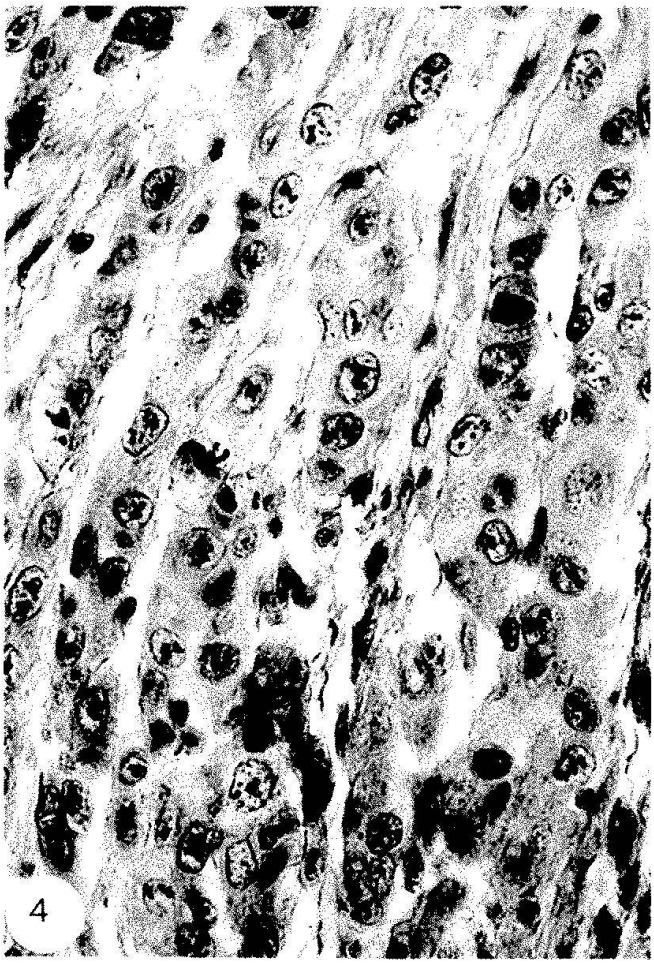

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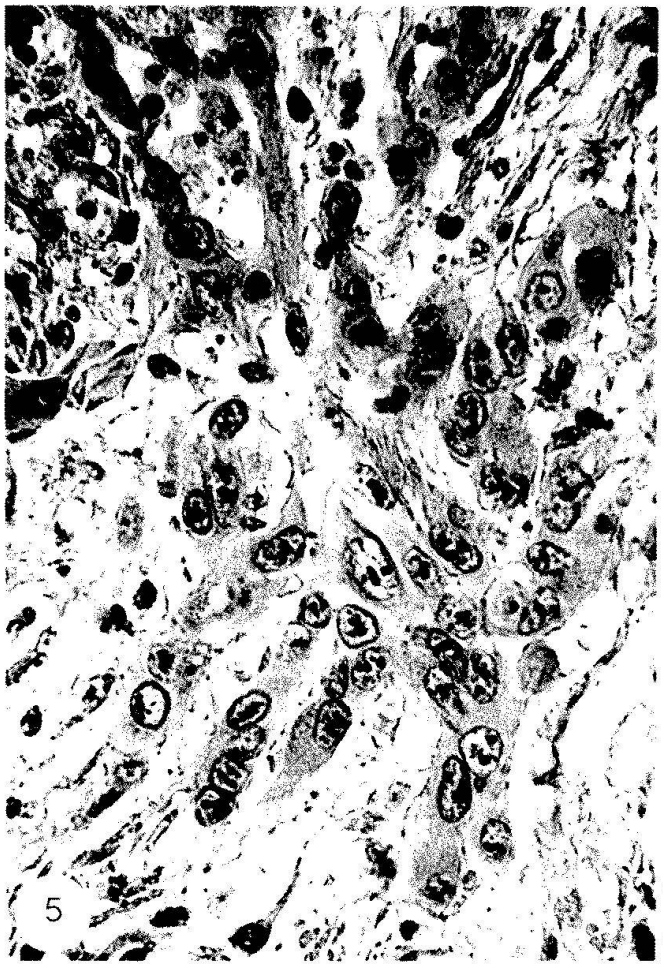

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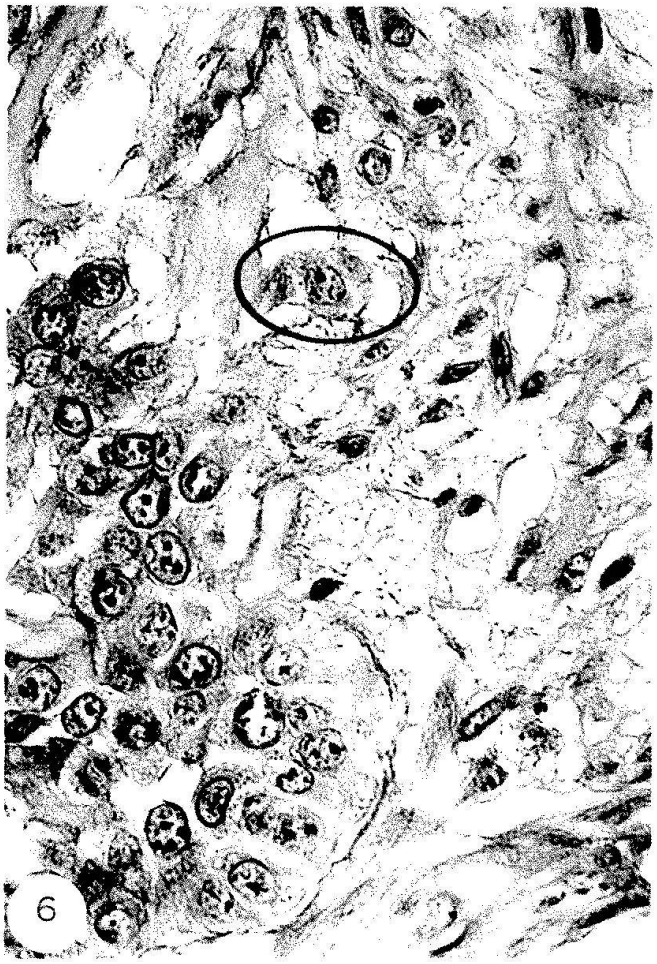

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few days, either by tumor growth in parallel sheets which split up and compress the stroma (fig. 4), or by the extension of tumor tongues which squeeze into stromal interstices (fig. 5). With progressive dissociation of the invasion front, single V2 cells and small cell groups are frequently found in advanced positions (fig. 6). Serial sectioning proves that in many instances such tumor elements are genuinely isolated and must have reached their acual sites by active translocation.

Lytic effects become recognizable within the first days after tumor implantation. They are exemplified in the next four figures which show the destruction of the skin muscle layer. Histological evidence indicates that this structure together with its collagen fascia perishes in contact with tumor cells (figs. 7, 8), tumor and host cells (granulocytes, monocytes, lymhocytes, fig. 9), and occasionally with host cells alone (fig. 10). Indeed, the studies of our group (Graf et al. 1981; Baici et al. 1982) have revealed that destructive effects in the invasion zone of the V2 carcinoma result from a kind of biochemical interaction between tumor and host with proteolytic enzymes from both sides as effectors.

As concerns the third mechanism of invasion, tumor cell locomotion, histology provides indirect evidence only for its occurrence and significance. Direct evidence is exclusively obtained by time lapse recording of cell motility. We have therefore studied the motile behavior of V2 carcinoma cells by microcinematography (Haemmerli et al. 1982) utilizing experimental conditions of increasing complexity: Motility on glass, on the surface of normal explanted rabbit mesenteries, and on and within mesenteries of rabbits which had received intraperitoneal implants of the carcinoma. From small tumor fragments explanted in culture chambers (figs. 11–15), single V2 cells detach, flatten, and migrate outwards under display of conspicuous leading lamellae. The migration of the cells in always short-distanced. Apparently the V2 cells, in spite of being on the move almost incessantly, have a tendency to stay in the vicinity of other cancer cells. This "socio-locomotory" behavior is consistent with the distribution pattern of single tumor cells in sections. The mesentery as a transparent membrane allows the microcinematographic recording of V2 cells translocating within a living tissue (figs. 16–19). As long as the tumor cells are on the surface of the mesentery, they move around in a similar configuration as on glass (figs. 16, 17). Penetration into the interior, however, requires shape adaptations, and V2 cells perambulating within the fibrillar meshwork of the mesentery display incessant and striking shape changes apparently imposed by the texture of the microenvironment (figs. 18, 19).

Recent studies on spread of the V2 carcinoma in the mesentery (Sträuli et al., 1983) provide new insight into the possible combinations of the three major mechanisms of invasion. Tumor cells that have migrated singly into the interior often begin to divide and thus give rise to small foci. A graded zone of complete and incomplete destruction of the loose connective tissue extends around such tumor nodules. Apparently, lytic effects depend on the close association of a minimal number of cells. Multiplication of V2 cells with formation of foci can also oc-

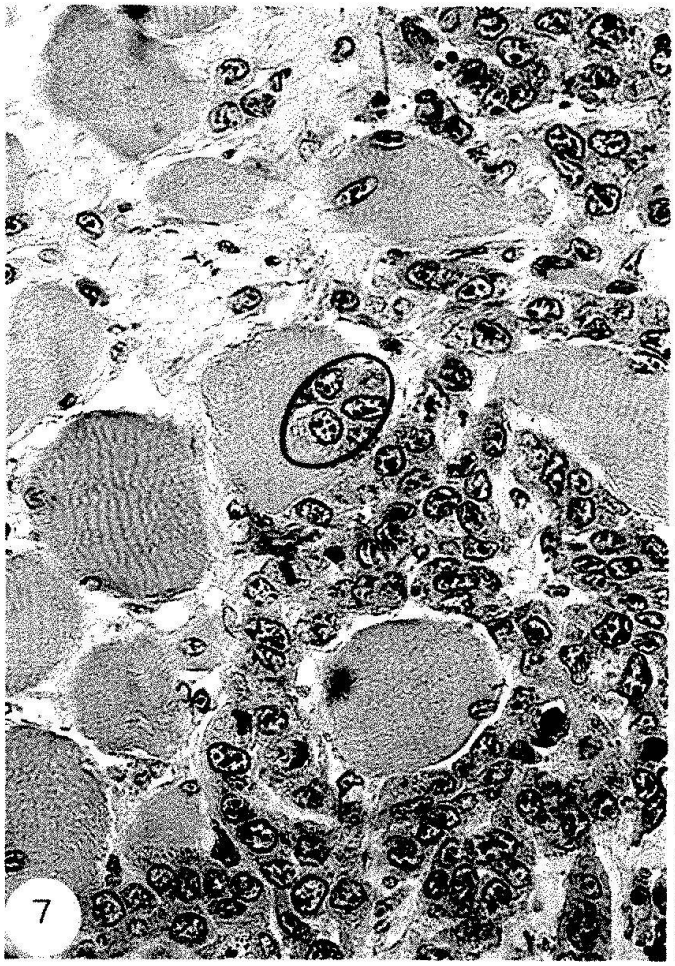
Figs. 7–10: Destruction of the skin muscle layer of the rabbit during invasion ot the s.c. implanted V2 carcinoma.

Fig. 7. The tumor (right and bottom) has invaded, isolated and partly destroyed the large fibers of the striated muscle. Penetration of carcinoma cells into a muscle cell is indicated by a circle. Magnification × 400.

Fig. 8. Three muscle fibers are invaded by carcinoma cells. While two fibers are completely replaced by the tumor, one fiber contains a rest of the sarcoplasma (arrows). Magnification × 400.

Fig. 9. Damage of muscle fibers decreases with distance from the tumor. The figure shows from left to right destruction, damage, and preservation of the musculature. Remnant of a muscle fiber is indicated by a circle. Magnification × 500.

Fig. 10. Destruction of the muscle layer by inflammatory host cells. The tumor (bottom) has reached and partly attenuated the collagen fascia (arrows) of the skin muscle. Host cells, apparently attracted by the tumor, have displayed their proteolytic capacity before reaching their chemotactic target, the carcinoma cells. Magnification × 500.



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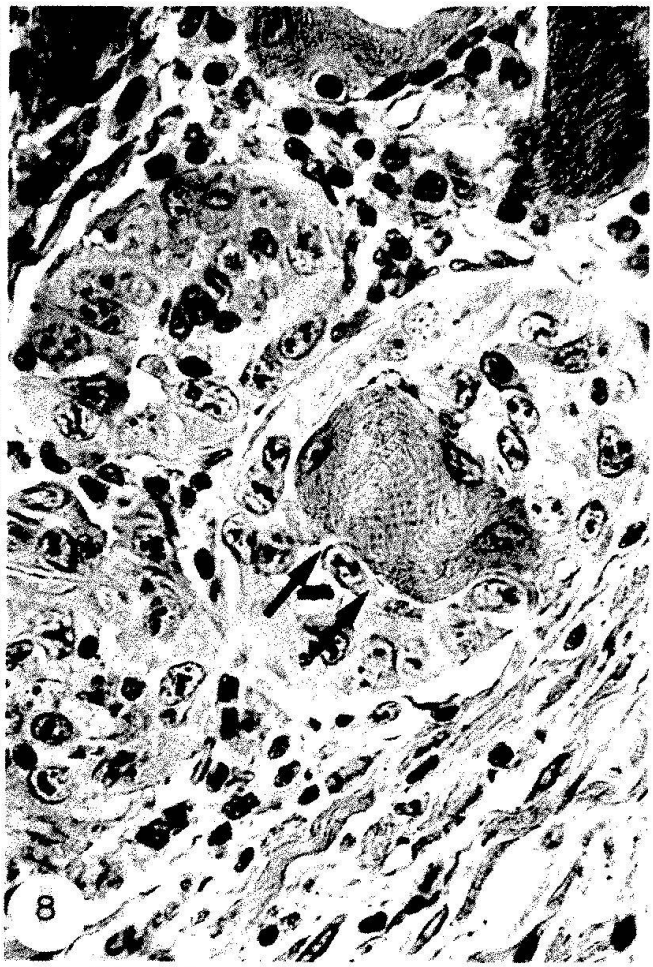

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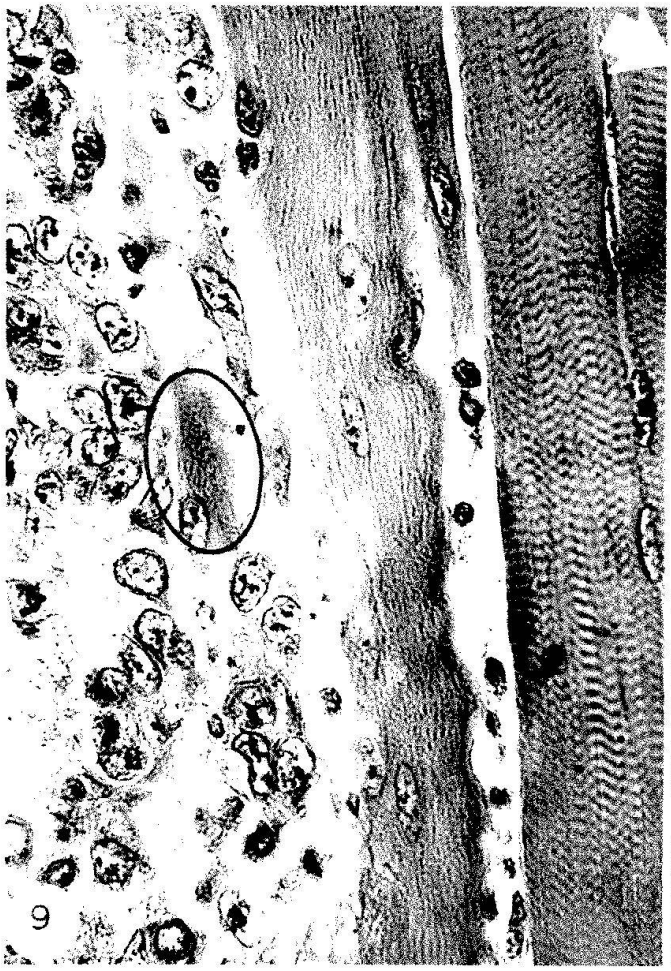

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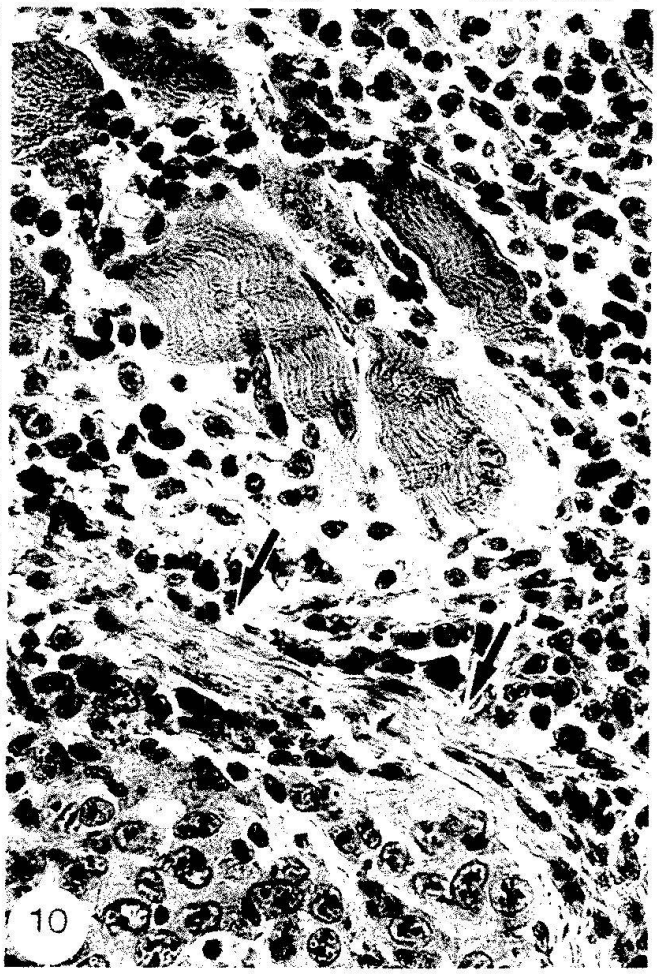

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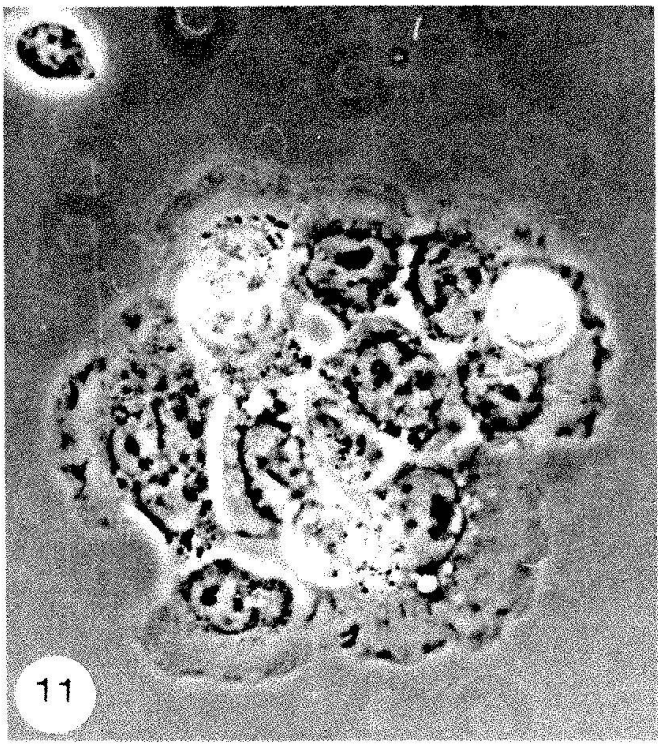
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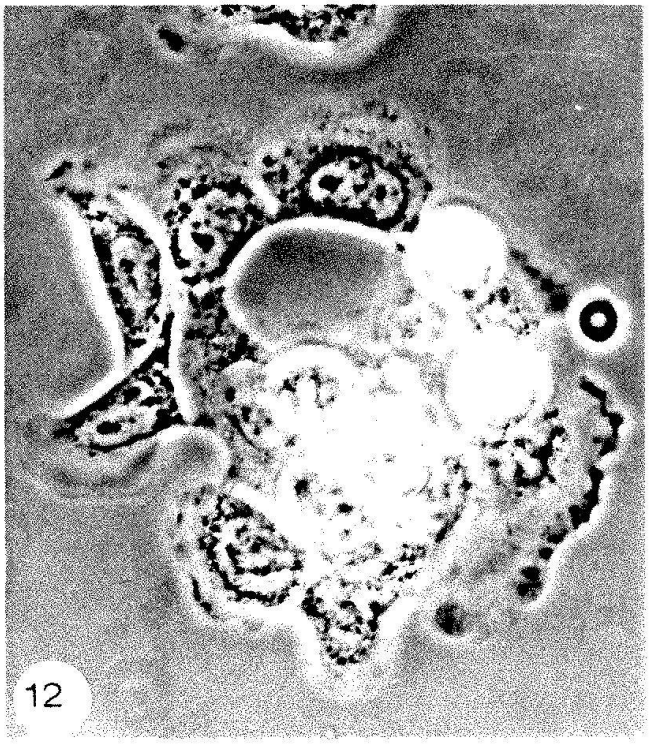
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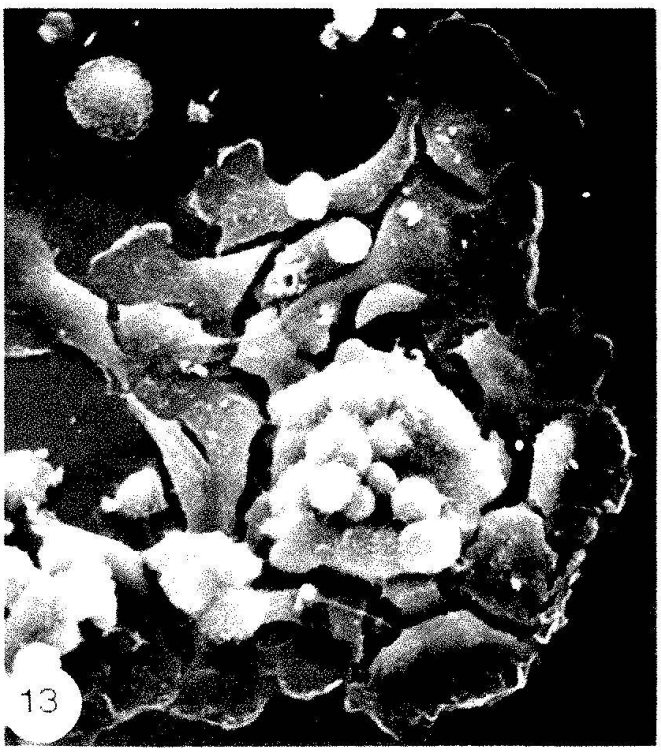
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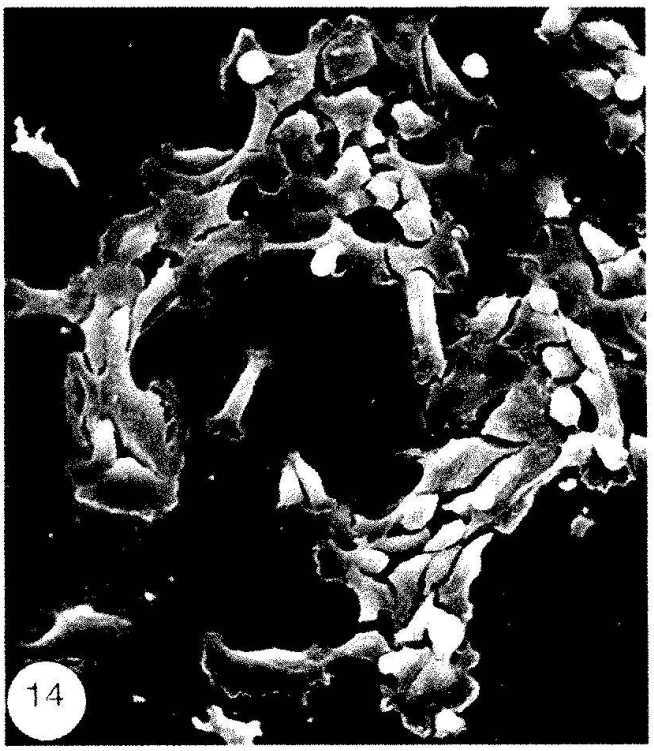
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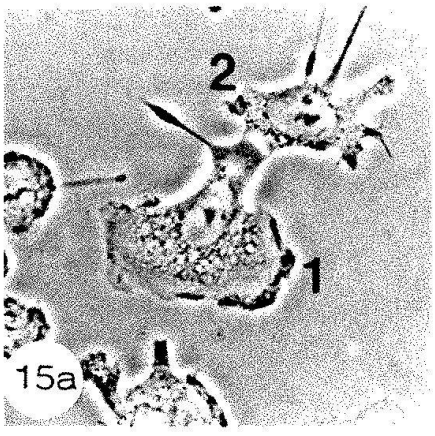
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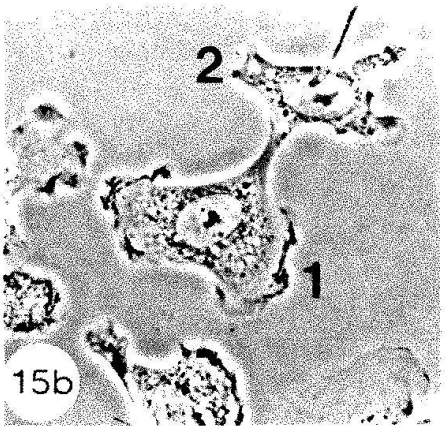
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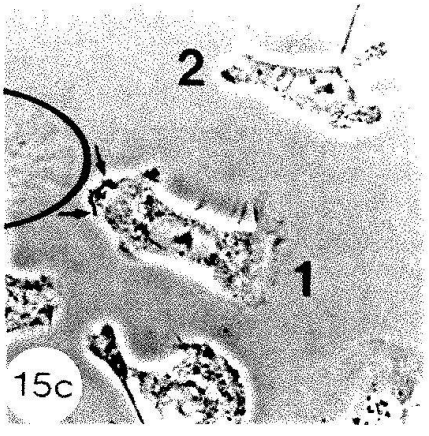
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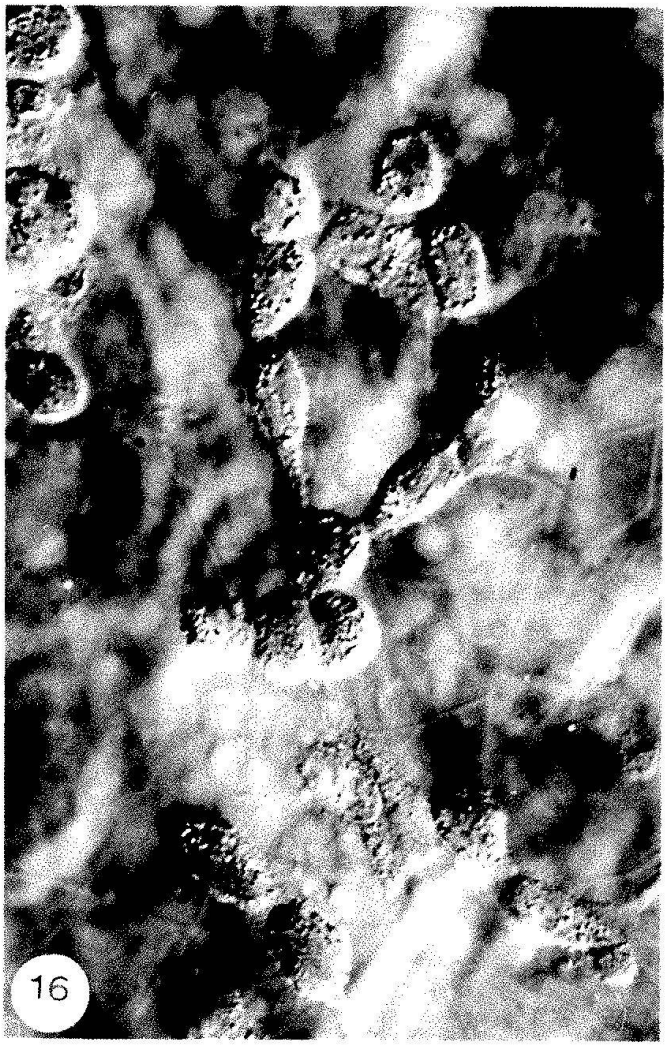
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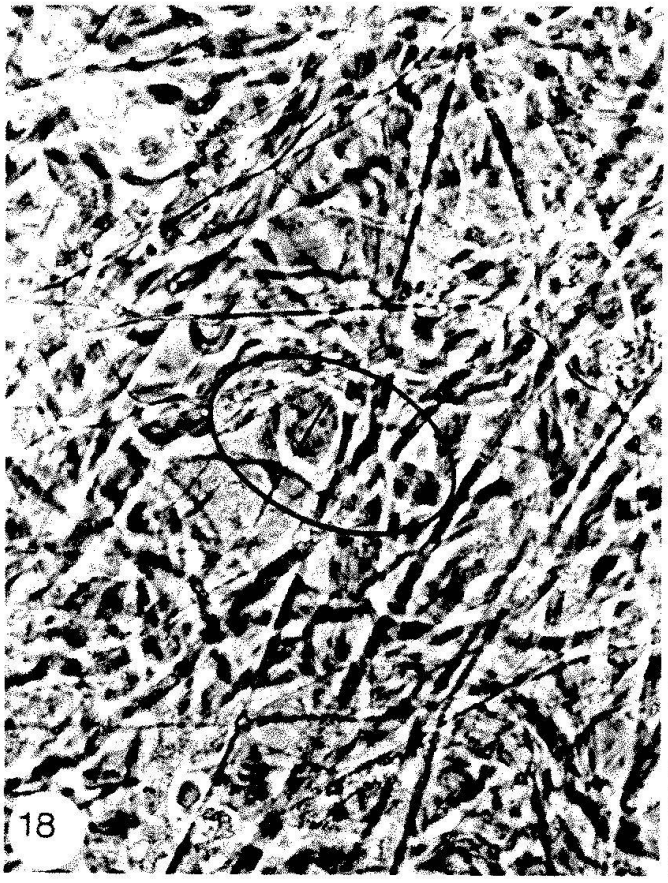
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cur at the surface of the mesentery. Such aggregates, preceded by a destruction zone, extend in depth and reach the interior, where single V2 cells get loose and, after short migrations, produce new tumor foci. It is conceivable that the invasive maneuvering of V2 cells at a given site and time is governed by environmental influences, but no such interdependence is clearly recognizable at the time being. At any rate, it appears that the tumor disposes always of a complete invasive potential: The V2 carcinoma is an absolute invader.

6. Concluding remarks: Progression of tumor invasion

As presented here for squamous cell carcinoma, a trend towards increasing invasiveness is disclosed by the combined engagement of clinical and experimental pathology. This trend fits well into the concept of tumor progression, but so far, the latter's connection with invasive potential is more descriptive than analytic. What causes the progression of tumor invasion? A discussion of the possible mechanism(s) is beyond the scope of this communication, but two major alternatives should be mentioned:
(1) The full potential of invasion is induced in the first cancer cell(s) by the transforming influences. The whole progeny of the transformed cell(s) is genetically primed for invasion, but expression of the potential depends on extragenetic factors, e.g. the size of the cancer cell population.
(2) The acquisition of the full potential of invasion depends on additional (posttransformational) changes of the genotype occurring either in the whole cancer cell population or in subpopulations, which may but must not coincide with clones or stem cells. Whether or not subpopulations are the carriers of the invasive potential is of minor importance for the fundamental aspects of the problem.
The host can be considered to be a decisive factor for both alternatives, and it is an intellectual and technical challenge to investigate in depth the impact of its anti- and pro-neoplastic activities on progression of tumor invasion.

Literature

Baici, A., Graf, M., Gyger-Marazzi, M. 1983: The V2 carcinoma of the rabbit as a model for tumor invasion: 3. Proteolytic activities. In: Tumor Progression and Markers. Proc. 6th Meeting Europ. Ass. Cancer Res., Budapest 1981, 47–50. Kugler Publications, Amsterdam.

Burghardt, E. 1973: Early Histological Diagnosis of Cervical Cancer. Thieme, Stuttgart.

Foulds, L. 1969: Neoplastic Development. Academic Press, London, New York.

Figs. 11–15. Motility of V2 carcinoma cells on glass.

Figs. 11–14. Dissociation of small tumor cell clumps placed on the lower glass surface of a cultur chamber. The cells migrate outwards, frequently under formation of arcade-like arrangements. Note the similarity of living cells (figs. 11, 12) and of cells fixed for scanning electron microscopy (SEM) (figs. 13, 14). Time interval: 1 hour. Phase contrast. Magnification × 300 (figs. 11, 12) SEM × 700 (fig. 13), × 350 (fig. 14).

Figs. 15a–c. Sequence from a time lapse film showing the detachment of a V2 cell (1) from another carcinoma cell (2). The membrane activity (arrows) displayed by cell no. 1 indicates the new direction of movement. Contact with the cell at the left side (circle) was established 10 minutes later. Time interval: 15 minutes each. Phase contrast. Magnification × 300.

Figs. 16–19. Motility of V2 carcinoma cells on and within the mesentery.

Figs. 16, 17. After injection of V2 tumor suspensions into the peritoneal cavity, the carcinoma cells adhere to the surface of the mesentery where they display their locomotory activity. Note the similarity in pattern formation shown in figure 16 to that of cells moving on glass (figs. 12 and 14). Single migrating V2 cells often show long posterior extensions (fig. 17). Differential contrast. Magnification × 600.

Figs. 18, 19. V2 cells migrating within the fibrillar meshwork of the connective tissue in the mesentery. Phase contrast. Magnification × 600.

Gittermann, C.O., Luell, S. 1973: Chemotherapeutic studies on metastasis of human epidermoid carcinoma (H.Ep.)3 in the embryonated chick egg. In: Chemotherapy of Cancer Dissemination and Metastasis, ed. by S. Garattini, G. Franchi, 279–291. Raven Press, New-York.

Graf, M., Baici, A., Sträuli, P. 1981: Histochemical localization of cathepsin B at the invasion front of the rabbit V2 carcinoma. Laboratory Investigation 45, 587–596.

Haemmerli, G., Arnold, B., Sträuli, P. 1982: Cell locomotion, a contributing factor in spread of the rabbit V2 carcinoma. International Journal of Cancer 29, 223–227.

Hamperl, H. 1959: Definition and classification of the so-called carcinoma in situ. In: Cancer of the Cervix (CIBA Foundation Study Group No. 3), 2–15. Churchill, London.

Kidd, J.G., Rous, P. 1940: A transplantable rabbit carcinoma originating in a virus-induced papilloma and containing the virus in masked or altered form. Journal of experimental Medicine 71, 813–837.

Koss, L.G. 1981: Pathogenesis of carcinoma of the uterine cervix. In: Cervical Cancer, ed. by G. Dallenbach-Hellweg, 111–142. Springer, Berlin, Heidelberg, New York.

Ober, K.G., Kaufmann, C., Hamperl, H. 1961: Carcinoma in situ, beginnendes Karzinom und klinischer Krebs der Cervix uteri. Geburtshilfe und Frauenheilkunde 21, 259–297.

Pietras, R.J., Roberts, J.A. 1981: Cathepsin B-like enzymes. Subcellular distribution and properties in neoplastic and control cells from human ectocervix. Journal of Biological Chemistry 256, 8536–8544.

Schiller, W., Daro, A.F., Gollin, H.A., Primiano, N.P. 1953: Small preulcerative invasive carcinoma of the cervix: The spray carcinoma. American Journal of Obstetrics and Gynecology 65, 1088–1098.

Seski, J.C., Schmidt, W.A. 1982: Growth patterns of microinvasive carcinoma of the cervix. In: Carcinoma of the Cervix, ed. by E.S.E. Hafez, J.P. Smith, 45–56. Martinus Nijhoff, The Hague.

Spornitz, U.M., Hafez, E.S.E. 1982: Morphology of the basement membrane in carcinoma of the cervix. In: Carcinoma of the Cervix, ed. by E.S.E. Hafez, J.P. Smith, 45–56. Martinus Nijhoff, The Hague.

Sträuli, P. 1980: A concept of tumor invasion. In: Proteinases and Tumor Invasion, ed. by P. Sträuli, A.J. Barrett, A. Baici, 1–13. Raven Press, New York.

Sträuli, P., In Albon, A.M., Haemmerli, G. 1983: Spread of the V2 carcinoma in the rabbit mesentery: Mechanisms of invasion and tumor-associated alterations of the extracellular matrix. Cancer Research 43, 5403–5410.

Toolan, H.W. 1954: Transplantable human neoplasms maintained in cortisone-treated laboratory animals: H.S.1; H.Ep.1; H.Ep.2; H.Ep.3; and H.Emb.Rh.1. Cancer Research 14, 660–666.

Willis, R.A. 1973: The Spread of Tumours in the Human Body. 3rd ed. Butterworths, London.

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Mechanisms in chemical carcinogenesis*

Werner K. Lutz

Abstract

In ever rising frequency, chemical substances are reported to have increased the tumor incidence in animal experiments. Although these compounds belong to a variety of chemical classes, a large number seems to have in common the ability to react with deoxyribonucleic acid (DNA), the carrier of genetic information, after metabolism to chemically reactive intermediates. As opposed to this group of genotoxic carcinogens, non-genotoxic carcinogens act by modulation of one or several out of a number of biochemical and biological steps governing amount and expression of unavoidable DNA lesions towards the formation of a tumor.

Zusammenfassung

Laufend wird über chemische Substanzen berichtet, von denen eine krebsfördernde Wirkung im Tierversuch gezeigt worden ist oder für den Menschen vermutet wird. Solche Substanzen kommen aus den verschiedensten Stoffklassen. Eine grosse Gruppe von organischen, schlecht wasserlöslichen Kanzerogenen wird über chemisch reaktive Zwischenprodukte metabolisiert. Deren Reaktion mit der Erbsubstanz DNS scheint das zentrale Element der Wirkweise von genotoxischen Substanzen zu sein. Da auch endogene, essentielle und unvermeidliche Verbindungen diese Eigenschaft haben, kann ein gewisses Mass an DNS Schäden nicht vermieden werden. Die Folgen werden allerdings dadurch gemildert, dass effiziente Reparatursysteme solche Schäden reparieren können. Die Wirkung nicht-genotoxischer Kanzerogene basiert auf der Modulation unvermeidlicher DNS-Veränderungen. Dies kann auf verschiedenen Stufen geschehen, z.B. durch Erhöhung der DNS-Bindung von anderen, genotoxischen Substanzen, durch Erhöhung der Ausbeute an kritischen DNS-Schäden, oder durch Beschleunigung des langsamen Prozesses der Entwicklung einer transformierten Zelle zu einem Tumor.

*Updated and modified version of the article in German "Mechanismen der Krebserzeugung", Neue Zürcher Zeitung Nr. 173, S. 39, July 29, 1981.

It was not necessary in the last years to read scientific journals to know that an ever increasing number of chemicals has been found to have increased the tumor incidence in animal experiments. In newspapers we were warned of *aflatoxins* on mouldy food; cigarette *smoking* is surely responsible for most lung cancers and is a contributing factor to many other types of cancer; *nitrosamines* form an important class of strong carcinogens which can be generated by nitrosation of amines; some *metal* salts have been discovered as industrial carcinogens and *hormones* were widely discussed very recently. *Asbestos* is an important factor in the induction of tumors from exposure at the work place, and *Saccharin* gave rise to headline news because of some bladder tumors induced in male rats whose diet consisted of 5 percent saccharin for life. In this introductory article I will not discuss the importance of these carcinogens for human health but I would like to present the current view on the mechanism of carcinogenic action of these chemicals. The structural formulas are given in figure 1. For cigarette smoke, benzo(a)pyrene is shown as a representative of the many carcinogens present. For the hormones, the synthetic estrogen diethylstilbestrol was taken.

No common feature can be discerned which could give a hint for some common mechanism of carcinogenic action of these compounds. More meaningful and perhaps more informative might be to look at the fate of these compounds in the animal.

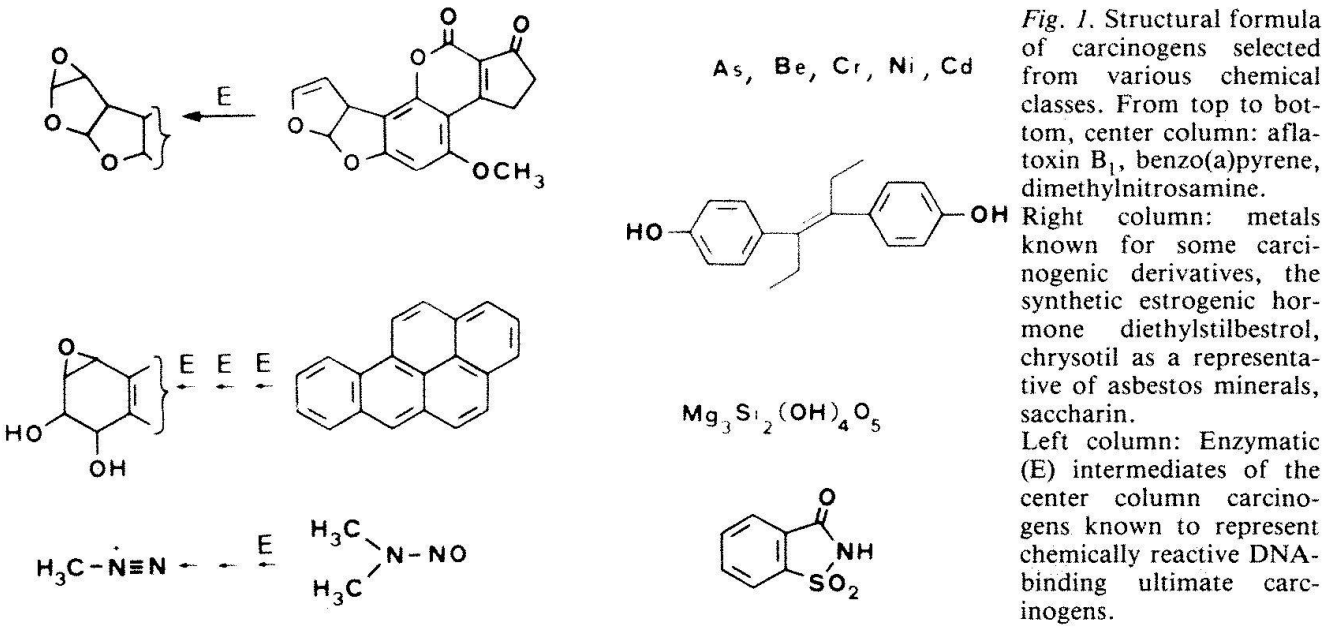

Fig. 1. Structural formula of carcinogens selected from various chemical classes. From top to bottom, center column: aflatoxin B_1, benzo(a)pyrene, dimethylnitrosamine. Right column: metals known for some carcinogenic derivatives, the synthetic estrogenic hormone diethylstilbestrol, chrysotil as a representative of asbestos minerals, saccharin. Left column: Enzymatic (E) intermediates of the center column carcinogens known to represent chemically reactive DNA-binding ultimate carcinogens.

Genotoxic carcinogens

Chemically reactive metabolites

One important aspect in the metabolism of many organic carcinogens is the fact that an excretion of the water-insoluble compounds is possible only after introduction of hydroxyl groups and subsequent conjugation with water-soluble molecules. In the course of these enzymatic oxidation processes, chemically reactive metabolites are formed, such as epoxides or diazonium ions as shown on the left hand side of figure 1. We have therefore found, at least for three of our standard carcinogens, the common feature of *electrophilic intermediates* which are also called ultimate carcinogens. These metabolites are unavoidably formed in a process that should finally lead to the excretion of the foreign compounds. By far the largest part is indeed rapidly inactivated by processes shown in figure 2, by rearrangements or enzymatic and non-enzymatic reactions with small molecules, and only a minute but biologically important fraction escapes and reacts with macromolecules, some of which are critical with respect to a triggering of the process of tumor formation.

DNA as critical reactant

There are many indications that most tumors have grown from one single cell. A heritable change must therefore have occurred in this cell. This is most directly achieved by some critical mutation in the genes (DNA). During cell division, DNA is replicated and evenly distributed among the two daughter cells. If a carcinogen is bound to the DNA, the copy process can be disturbed so that one daughter strand carries a wrong piece of information (fig. 3). This is called mutation and the mechanism of action of the carcinogen was by *genotoxicity*. By far not all carcinogens chemically bound to

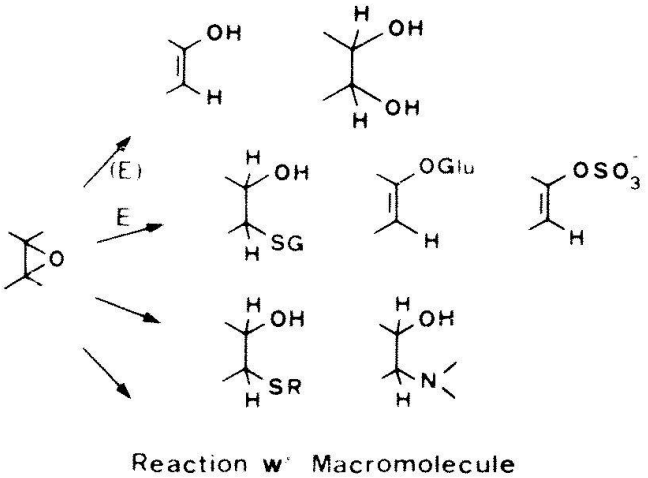

Fig. 2. Chemical and enzymatic (E) reactions involved in the further metabolism of reactive intermediates, such as epoxides. 1st row: rearrangement, addition of water. 2nd row: enzymatic conjugation reactions with glutathione (GS), glucuronide (Glu), sulphate. 3rd row: non-enzymatic reaction with low molecular nucleophiles, containing thiol and amino groups. 4th row: reaction with macromolecules, such as protein and nucleic acids.

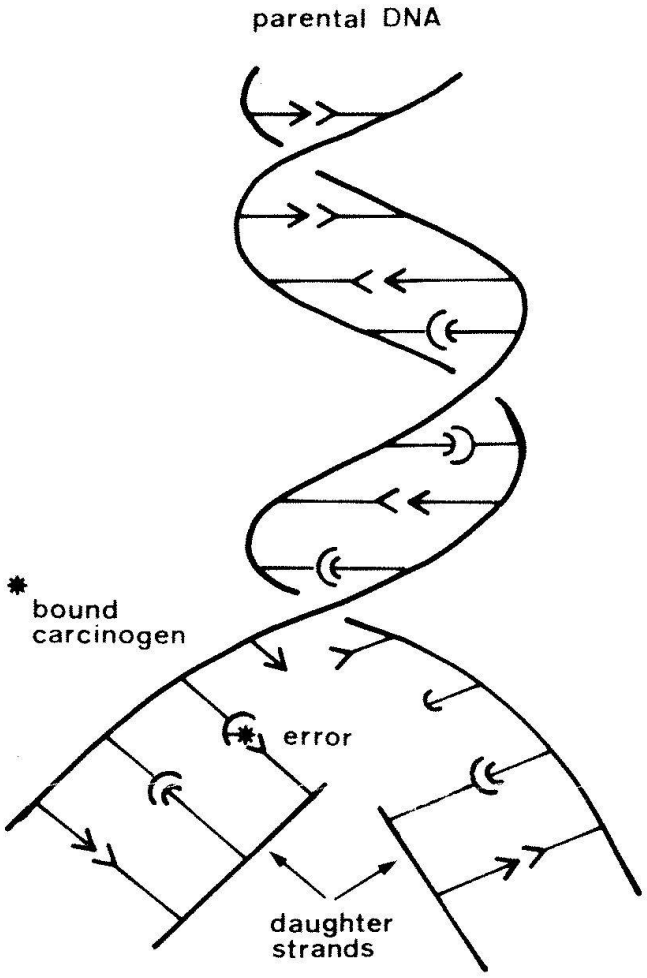

Fig. 3. Schematic representation of the deoxyribonucleic acid (DNA) genes, the heritage of a cell, during replication before cell division. The bound carcinogen molecule (*) can disturb the replication process in a way as to direct a wrong coupling partner to be introduced into the daughter strand. If this wrong piece of information lies in a critical gene, the cell might be initiated to become a cancer cell.

DNA produce a mutation. A number of processes are known for the *repair of the DNA*, and it is astonishing that microorganisms and phylogenetically lower animal species possessed such repair mechanisms long before the synthetic chemistry invented new genotoxic compounds.

Unavoidable DNA damage

We must therefore assume that DNA damage is as old as life and that some unavoidable genotoxicity resulted in a strong evolutionary pressure to develop efficient DNA repair systems. Among these unavoidable sources of DNA damage is radiation, both cosmic and terrestric gamma rays as well as UV. A variety of endogenous or essential compounds are degraded by the same routes of oxidation known for the genotoxic carcinogens. Many genotoxic carcinogens are produced in the process of cooking or frying and are not completely avoidable, and the formation of carcinogenic nitrosamines can take place in the acidic milieu of the stomach by nitrosation of ubiquitous amines in the presence of nitrite generated from bacterial reduction of nitrate. In addition, many genotoxic agents are of natural origin. Besides the well-known mycotoxins, such as aflatoxins, there are pyrrolizidines, widely distributed alkaloids in plants. Safrole and estragole are components of many spices, gyromitrin is a carcinogenic hydrazone derivative isolated from the false morel mushroom Gyromitra esculenta, and the next years will see the discovery of many more carcinogens of natural origin. There can therefore be no doubt that a certain level of DNA damage cannot be avoided.

Now that we have shown that the first three of our standard carcinogens act by genotoxicity, let us discuss the remaining. It has been shown with the carcinogenic *metal* ions that their presence during the replication of DNA decreases the fidelity in the synthesis of an exact copy for the daughter strands. We could therefore call this mechanism of action an *indirect genotoxicity*. What about the others?

Non-genotoxic carcinogens

In order to answer this question it might be helpful to summarize at this point the general knowledge of the process of tumor formation (fig. 4). We have already discussed the processes leading to the DNA binding of genotoxic carcinogens. The resulting, potentially critical DNA lesion can lead to the transformation of the cell to a tumor cell able to progress to a tumor. These final steps are often summarized under the term "promotion" a process which characteristically requires a substantial fraction of the animal's life time, whereas the earlier steps leading to the critical DNA lesion can take place in a few days.

Since we must assume that our DNA is constantly damaged to some unavoidable extent

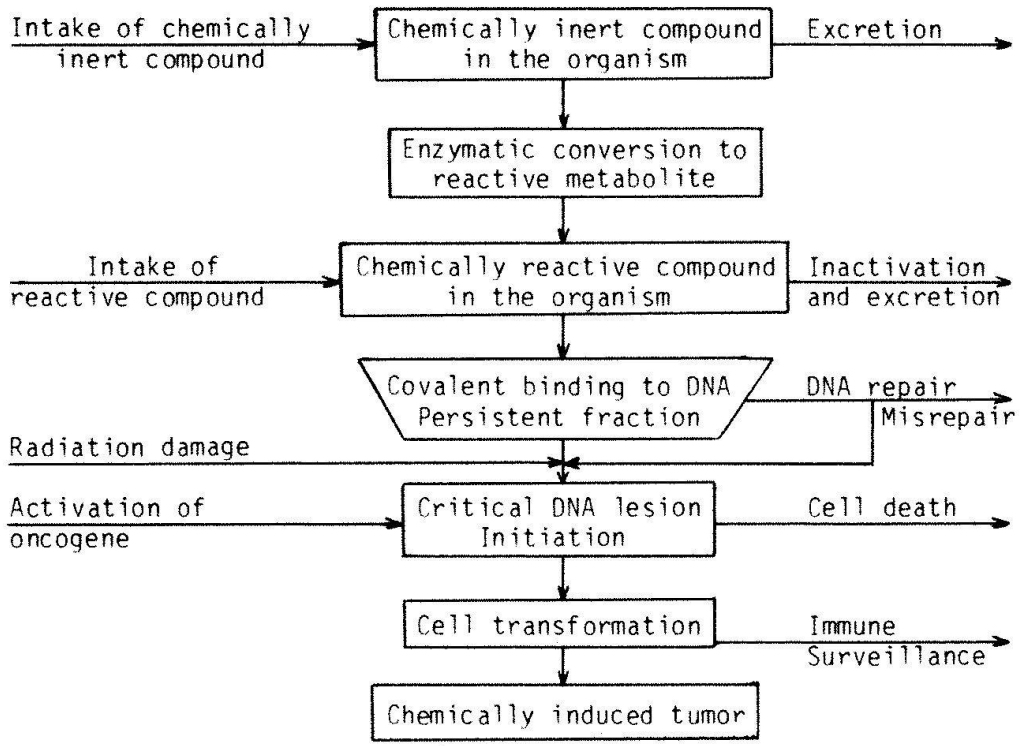

Fig. 4. Schematic representation of the sequence of events governing the process of tumor induction by chemicals (vertical axis). The horizontal arrows pointing to the right summarize some of the reactions protecting the host from the carcinogenic stimulus.

we can deduce that those compounds also increase the tumor incidence which increase the amount of DNA damage set by genotoxic substances or help to promote the frequency of cell transformation to a tumor cell and the progression to a tumor. In the flow chart shown in figure 4, this means that all chemicals which have an influence on the speed of any of the reactions will alter the final tumor incidence. Cocarcinogenesis is achieved by accelerating the vertical processes or slowing the horizontal rescue processes (to the right hand side), anticarcinogenesis is achieved by the opposite activity. For the sake of an easy classification, the site of modulation can roughly be subdivided into three parts.

Modulation of DNA binding

The first group of non-genotoxic carcinogens leads to an increase of the DNA damage by genotoxic carcinogens. One example for this type of activity might be the generation of nitrosamines from amines and nitrite in the stomach. Since this reaction is dependent on the pH, hyperacidity of the stomach might well be a modulatory factor, and a physiological basis for an eventual relation of stress factors with gastro-intestinal tumors might be envisaged.

Many studies deal with the influence of drug-metabolizing enzyme activities on DNA damage. In all the studies so far reported, a pretreatment of a laboratory rodent with an enzyme-inducing agent has resulted in a slight decrease of DNA binding by a subsequent dose of a standard carcinogen, such as benzo(a)pyrene. Because of the complexity of activating and inactivating processes, enzymatic and non-enzymatic, which govern the concentration of reactive intermediates, this finding should not, however, be taken as representative for other carcinogens. Situations will certainly arise where the induction of enzyme activities will result in a higher level of DNA binding exerted by genotoxic carcinogens.

Co-mutagenicity

The fixation of the primary DNA damage, the binding of a genotoxic carcinogen, in the form of a heritable mutation is a central event in chemical carcinogenesis and is subject to a number of important modulatory influences. After DNA binding by a genotoxic carcinogen a competition between DNA repair and DNA replication starts. All stimulation of cell division reduces the time allotted for repair and an increased fre-

quency of mutations can result. Cell division is an absolute requirement for growth and for the proper functioning of a number of tissues and there are endogenous stimulants for this type of response, such as *hormones*. The regenerative processes which are elicited after exposure to cytotoxic compounds or from local irritation could explain the carcinogenicity of implants or of insoluble *asbestos* fibers. It is interesting in this respect that lung tumor incidence is greatly enhanced for cigarette smokers exposed to asbestos. It is possible that the DNA damage exerted by the genotoxic constituents of cigarette smoke can be repaired less effectively if the cell division rate is increased in the presence of an asbestos fiber.

Promotional activity

For a discussion of the last steps and the respective modulations, some background knowledge is required: It is not as yet generally known what type of lesion is required to convey to a cell the attitude of a cancer cell. In some special cases there is indication for a differentiation back to some type of multipotent ancestor cell. In other systems, the activation of some oncogene has been shown to induce the transformation of the cell. An intriguing characteristic of the chemical induction of a tumor is the fact that a considerable fraction of the life is required in most situations. During this latency period of up to 20 years in man, of many months in the rat, there seems to be a requirement for the continuous challenge of the initiated cell by something called "promoter". The most potent promoters have been isolated from plants, and the classical constituent is a phorbol ester, a diterpene derivative isolated from the oil of croton seeds. This compound has been found to bring about a significant tumor incidence if painted repeatedly on the skin of mice after one single topical application of benzo(a)pyrene as genotoxic carcinogen. Although there is only insufficient evidence that tumor promoters are not by themselves genotoxic, such a mode of action seems unlikely. Tumor promoters have been found to induce a variety of biochemical and biological responses but it is not known which one is causally related to the effectiveness in cancer induction.

The search for a reliable short-term test on promoting activity of a compound is therefore very active these days, and there are reports that *saccharin* has been found to exhibit qualitatively similar effects like typical promoters. For this last compound selected from our headline news, we were able to exclude a DNA binding activity and it is possible that saccharin is extremely weakly active as a modulator of the final but long stages of tumor promotion and progression. There is good epidemiological evidence that fat consumption is correlated with the risk of cancer, especially of the colon and the breast. Animal experiments have shown that dietary fat can indeed increase the tumor incidence if given continuously after a single dose of a genotoxic carcinogen. In addition to this promotional type of activity, a genotoxicity of fat itself cannot yet be ruled out because it is well known that polyunsaturated fatty acids readily form chemically reactive derivatives, such as peroxides. Furthermore, there is evidence that the intestinal bacterial flora can play an important role in the generation of genotoxic carcinogens from non-carcinogens, e.g. by reduction of nitroarenes to carcinogenic aromatic amines. Since the diet ultimately determines the composition of the bacterial flora with respect to strain and number, it might well be that a fatty diet predisposes the host to carry potentially dangerous intestinal bacteria.

Multiple modulatory activities

The situation is therefore not as simple as I have depicted it in the introductory paragraphs. Just like fat, many carcinogens do not only act on one single level. Cigarette smoke is another well studied and illustrative example. It is well known that it contains a number of genotoxic organic chemicals, enzyme-inducing agents and also carcinogenic metals such as cadmium. The nitroxides and aldehydes present in smoke are cytotoxic and irritate the mucous membranes of the respiratory tract. The resulting synergism might be the reason why as much as about thirty percent of all tumors in man, non-smokers included, can be traced back to cigarette smoking.

Toxicological implications

On the basis of the above analysis, we can conclude that endogenous factors are operative on all levels of tumor induction so that cancer will never be completely avoidable. Evaluation of the epidemiological data available suggest that this unavoidable tumor incidence will lie somewhere between 10 and 30 percent, assuming that the life expectancy remains unchanged. In order to achieve this low risk for ourselves, it would be most profitable to avoid all those exogenous factors that act strongly on many levels. Above all, *cigarette smoking must be stopped.* Then, it seems that a *reduction of fat consumption* and an *increased uptake of undigestable fibers,* possibly in the form of vegetables and fruits rich in vitamins, will have a beneficial effect. And finally, it will be beneficial to reduce the uptake of known and unknown carcinogens with the diet by *eating little of everything.* These simple rules will much more effectively result in a decreased cancer incidence in the general population than any other cancer policy.

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Induction of endogenous virogenes and oncogenes in the pathogenesis of leukemia

Christoph Moroni

Introduction

Oncogenic retroviruses fall into two classes, those with slow and those with acute transforming ability. The former are termed leukemia viruses; they induce leukemia after about 3–12 months following virus inoculation. The latter are termed sarcoma- or acute leukemia viruses. Their effect becomes obvious after 1–2 weeks after inoculation. They transform fibroblasts and other suitable target cells *in vitro*. This is in contrast to the leukemia viruses which do not display transforming activities *in vitro*. The important difference between the two classes of viruses is that the sarcoma viruses, but not the leukemia viruses, carry additional genes, called oncogenes, which control neoplastic transformation. Interestingly, these oncogenes are normal cellular host genes which have found their way into the viral genome via recombination. In other words, sarcoma viruses have evolved from non-transforming retroviruses by recombination with host oncogenes. It is the "picked up" oncogene which controls and directs the malignant phenotype (for references and review see Weiss et al., 1982). In vertebrate cells, the oncogenes are thought to carry out normal functions of still unknown nature, while under the control of the viral genome they cause transformation.

The first mammalian leukemia virus was isolated by Gross from AKR mice (Gross, 1951). It was only 20 years later that it was realized that all mice carry in their genomes multiple copies of integrated retroviral genomes, collectively called endogenous viruses. Nucleic acid hybridisation ("Southern blot") analyses shows that mice carry about 15–25 viral copies related to the Gross leukemia virus. They are inherited as normal cellular genes, following Mendel's laws and appear to be subject to normal gene regulation by the host (Aaronson and Stephenson, 1977). They offer a model to study gene expression in eukaryotic cells.

There is heterogeneity amongst endogenous viruses. (In this article, we deal only with the endogenous viruses related to leukemia viruses). Many copies show deletions and are therefore defective. When expressed, they may lead to production of viral proteins, but not to infectious virus. The complete infectious viruses of inbred mice fall into two groups, distinguishable by their host range. Xenotropic viruses replicate in non-mouse, e.g. rabbit cells, but not in mouse cells (Levy, 1973; Levy, 1978), while ecotropic viruses show the reciprocal behaviour (Pincus et al., 1971). There is recombination amongst endogenous viruses. In leukemogenesis, an ecotropic virus recombines with a (presumably defective) virus related to the xenotropic group to generate a dual-tropic virus which replicates both in mouse- and non-mouse cells (Hartley et al., 1977).

In retroviral leukemogenesis the following steps can be distinguished: First, there is expression and high-titer replication of ecotropic viruses. This can be achieved by spontaneous induction of the ecotropic viral loci as in AKR mice, or, when these loci are silent or absent, by passive inoculation of an ecotropic viral stock. In AKR mice, later in life, the endogenous xenotropic virus becomes expressed, and with the onset of the characteristic T-cells thymoma the dual-tropic virus appears (Rowe et al., 1980). It may be that only this latter virus has the ability to infect the target cell for neoplastic transformation. It is thought that the long latency of this disease is related to the time required to generate the dual-tropic virus and to infect the appropriate target cell. How does a virus lacking known transforming genes induce transformation? From work with avian leukosis virus, which also lacks

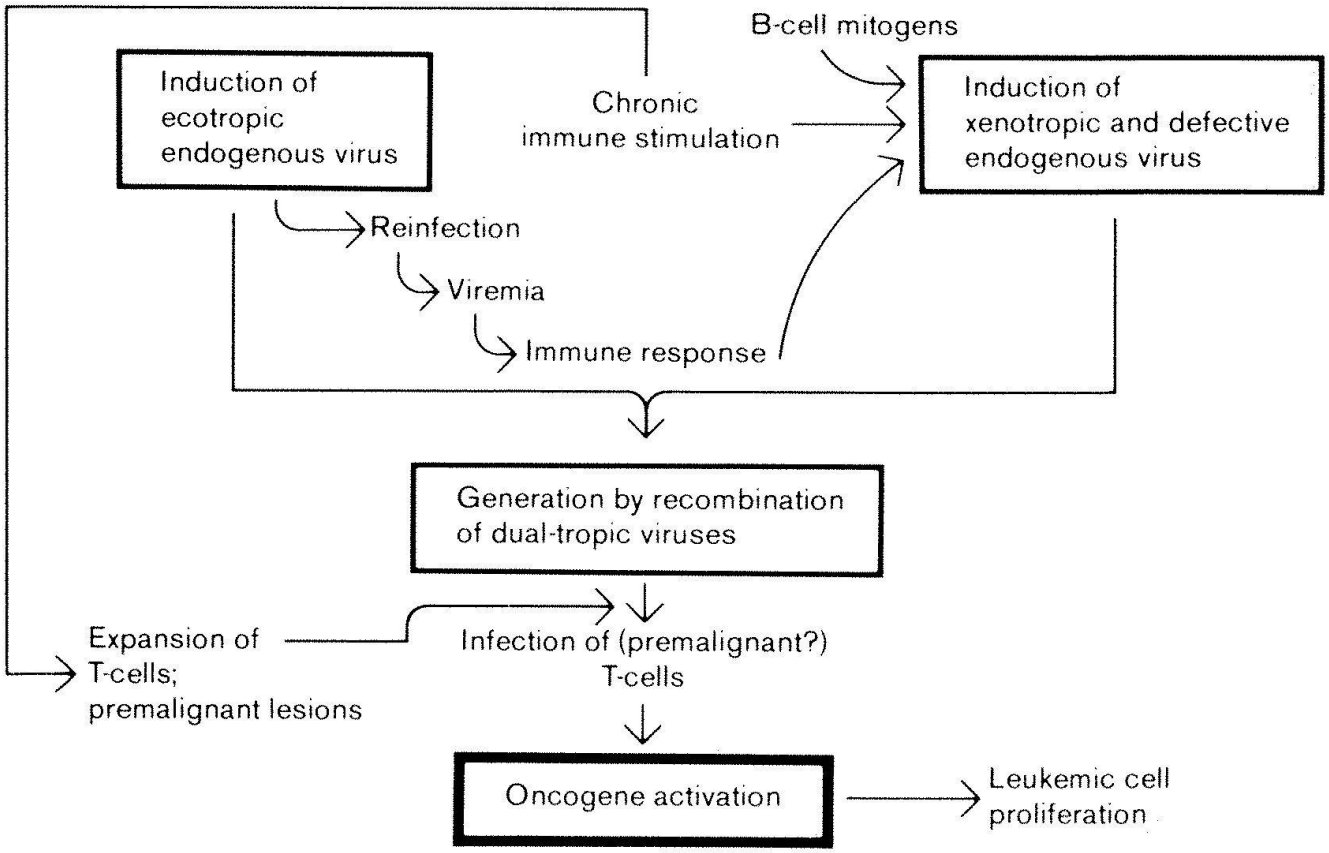

Fig. 1. Minimum model of retroviral leukemogenesis

oncogenes, we know that following infection viral DNA can integrate next to a cellular *onc* gene, in this case the *myc* gene (Hayward et al., 1981). Viral control elements called LTR (see Diggelmann, this volume) containing promoter sequences can then induce the transcription of the neighbouring *myc* gene. It may be that in murine leukemia, the dual tropic virus induces an oncogene in a T-cell in the way avian leukosis virus does in the B-cell tumor of the chicken. Figure 1 shows a "minimum model" of leukemogenesis. According to this model, the critical steps are: induction of at least two different endogenous viruses; the recombinational generation of dual-tropic viruses; and, the activation of oncogenes. Chronic immune-stimulation, known to favor leukemogenesis, may affect this process by two ways: by inducing endogenous viruses, and by triggering pre-malignant changes in T-lymphocytes. There is some evidence that such changes are even required to allow T-cell infection to take place; normal T-cells which grow *in vitro* cannot be infected by retroviruses (Horak et al., 1981; Stoye and Moroni, unpublished results).

Induction of endogenous viruses

The multiple copies of endogenous viruses are integrated at different sites and apparently in many chromosomes. It appears that they have been acquired by exogenous infection, and have succeeded in maintaining themselves in the genome of the species. Over time, they have duplicated, changed their position in the genome (perhaps by reinfection of the germ line) and have given rise to the heterogeneity observed to date. It is not known why they have been maintained for long periods of time. Either they offered to their host a selective advantage of unknown nature, or they found an ecological niche as "harmless" molecular parasites, causing rare leukemias and sarcomas but not affecting the viability of the species as such. As expected, the host has evolved protection mechanisms to prevent reinfection of spontaneously induced viruses. Two such mechanisms are known: First, there is a serum lipoprotein which is highly and selectively active in neutralizing xenotropic virus. Second, mouse cells lack receptors for xenotropic virus and therefore cannot be rein-

fected once virus has become activated (Levy et al., 1975; Levy, 1978). Reinfection by ecotropic virus is restricted by an intracellular mechanism which prevents integration of viral DNA into the host genome and is controlled by the host Fv-1 locus (Lilly and Pincus, 1973).

The induction of endogenous viruses is important for two reasons. First, it corresponds to one step in the development of retroviral leukemia. Second, it is of interest in the general context of induction and function of eukaryotic genes. The analysis of virus-induction is complicated by the heterogeneity mentioned above. If one wishes to make statements about the induction of a single provirus, care must be taken to exclude the induction of additional loci. This heterogeneity also has its merits: viruses integrated at different sitey may serve as guides and probes to chromosomal regions for which no marker yet exists.

In the following sections some pertinent facts about induction of xenotropic, ecotropic and defective viruses are being summarized.

a. Xenotropic viruses

Some strains of mice (prototype NZB) spontaneously produce high titers of xenotropic viruses. A genetic analysis revealed that they carry two non-linked loci, Nzv-1 and Nzv-2, which control virus production (Datta and Schwarz, 1977). It is not known whether the lack of virus-repression results from a specific integration site, or from sequences present in the viral genome. Genetic information for a different type of xenotropic virus is present in most strains of mice, but expression is repressed. When fibroblasts from such strains, e.g. BALB/c, are treated with IrdU or BrdU, production of the repressed xenotropic virus is induced in some of the cells (Lowy et al., 1971; Aaronson et al., 1971). The mechanism for this induction is still poorly understood. Suboptimal doses of IrdU followed by UV treatment lead to virus induction, suggesting that chromosome brakes may be involved (Teich et al., 1973). Other agents which have been found to be virus-inducers in fibroblast cultures include inhibition of protein synthesis (Aaronson and Dunn, 1974), L-canavanine (Aksamit and Long, 1977), hydroxyurea (Rascati and Tennant, 1978) and 5-azacytidine (Groudine et al., 1981; Niwa and Sugahara, 1981). We have been interested in the induction mechanisms operating in lymphocytes which are the target cells of leukemogenesis. We found that induction of xenotropic virus occurs in cells during B-cell differentiation (Moroni and Schumann, 1975; Moroni et al., 1978). This can be shown by culturing spleen-of lymphnode cells *in vitro* with B-cell mitogens, such as bacterial lipopolysaccharides, lipoprotein or tuberculin, which all trigger B-cell differentiation. Induction was enhanced by BrdU (Moroni et al., 1975). There is evidence that virus induction is linked to the process of differentiation itself: 1. When B-cell mitogens, such as dextran sulfate, lacking the capacity to induce terminal differentiation are used, no induction is seen (Moroni and Schumann, 1978). 2. CBA/N mice show a recessive sex-linked defect in B-cell differentiation. When F_1 animals in a cross with wild type mice are examined individually, only the male animals exhibit B-cell differentiation and virus-induction (Phillips et al., 1977). 3. LPS-induced B-cell differentiation can be blocked by prior treatment of B-cells with anti-μ serum. When this is done, virus induction is also impaired (Stoye and Moroni, in preparation). In a genetic cross (Stoye and Moroni, 1983) involving inducible BALB/c mice and non-inducible 129 mice, induction by mitogen as well as the BrdU-amplification effect segregated as a single trait, closely linked if not identical to the locus Bxv-1 which controls virus-induction by fibroblasts, and was discovered by Kozak and

Table 1. Cell type specificity of the induction of xenotropic and ecotropic endogenous viruses

	Xenotropic virus			Ecotropic virus		
	B-cell	T-cell	Fibro-blast	B-cell	T-cell	Fibro-blast
B-cell mitogen	ind.	—	—	—	—	—
T-cell mitogen	—	—	—	—	—	—
BrdU	ampl.	—	ind.	—	—	ind.

ind.: induction
ampl.: amplification
—: no effect

Rowe (1980). In our cross, we indentified a second inducible locus (Bdv-1), unlinked to Bxv-1, which controls the induction of a defective virus (see below), and is also induced by LPS (Stoye and Moroni, 1983). We view Bxv-1 and Bdv-1 genes as viral markers integrated in those regions of the chromosome active in B-cell differentiation. It will be interesting to learn what cellular functions are encoded in the DNA adjacent to these viral loci.

b. Ecotropic viruses

Mice carrying ecotropic viruses fall into two groups. Some (AKR, C58) produce virus spontaneously, which leads later in life to leukemia. Other mice (BALB/c, C57Bl/6) do not express virus, but can be induced to do so by *in vitro* treatment of their fibroblasts by IrdU. Induction is controlled by a single dominant locus (Stephenson and Aaronson, 1972) mapping on chromosome 5 (Kozak and Rowe, 1979). Interestingly, no ecotropic virus is induced in B-cells under conditions that induce xenotropic virus. Table 1 summarizes the induction pattern for ecotropic and xenotropic viruses. Virus production appears to be virus type- and cell typespecific and the observed patterns raise interesting questions on the relationship between the differentiation stage of a cell and virus expression.

c. Defective viruses

Most endogenous viruses are defective and their analysis is difficult in the absence of provirus-specific assays. Eventually, monoclonal antibodies or specific DNA-probes may provide the necessary tools. The most challenging question at present is to identify and induce the (presumably defective) virus which is involved in the recombinational event which generates the leukemogenic dual-tropic virus. We have demonstrated the induction of a defective virus from strain-129 mice which lack inducible replicating viruses. With BALB/c mice, we have shown that viruses can be induced from B-cells. The first is the xenotropic virus mentioned above, the second is a defective but reverse transcriptase-positive virus. In a genetic analysis, the two loci were found to segregate (Stoye and Moroni, 1983).

While T-cells cannot be induced to produce complete virus, incomplete virus expression from what appears to be defective endogenous genomes has been observed. Thymus T-cells, for example, express constitutively an antigen (G_{IX}), which corresponds to the retroviral gp70 protein (Tung et al., 1975). When mature T-cells are activated by concanavalin A, viral gp70 antigen becomes induced and inserted into the cellular membrane. This can be demonstrated by the fact that activated, but not resting T-cells can be killed by anti-gp70 antibody in the presence of complement. Interestingly, gp70-induction was shown on different subpopulations of T-cells, namely T-helper, T-suppressor, and cytotoxic T-cells (Wecker et al., 1977; Wecker and Horak, 1982; Klenner et al., 1982). As argued above for B-cells, it appears that the induced provirus in activated T-cells lies in a chromosomal region important and perhaps specific for T-cell maturation.

Using a fluorescence-activated cell sorter, Morse et al. (1979) found a gp70 molecule related to the xenotropic virus on lymphocytes of all the strains they tested. The relative amounts in different organs varied in a strain-specific way; induction of this defective virus is under host control. In conclusion, different proviral loci become expressed during the different phases of lymphocyte differentiation. This in turn may favor the recombinational event generating the leukemogenic variants and explain the observed association between leukemogenesis and hyperblastic dysfunctioning of the immune system as observed following graft-versus-host reactions (Schwartz and Beldotti, 1965).

Oncogene activation

Following infection by sarcoma- and acute leukemia viruses, the viral oncogene becomes expressed and directs malignancy. After it was realized that viral oncogenes are transduced host genes, the question arose wheter these cellular host "oncogenes" – more appropriately called proto-oncogenes – might also become activated and play a role

in non-viral malignancies. Recent evidence suggests that this may be the case.

The oncogenes known at present fall into two, partially overlapping groups. The first group contains the oncogenes present in sarcoma or acute leukemia viruses. Examples are the *src* gene of Rous sarcoma virus, *myc* of avian myelocytomatosis virus, *mos* of mouse Moloney sarcoma virus. The second group contains genes which have been identified by their ability to induce foci of transformed cells following transfection into suitable fibroblast cells (NIH 3T3 cells). Many human tumor lines, but also primary human tumors contained oncogenes, as revealed by transfection experiments, amongst them lung-, colon-, bladder-mammary-tumors and leukemias (Shih et al., 1981; Murray et al., 1981; Lane et al., 1981; Lane et al., 1982; Perucho et al., 1981; Pulciani et al., 1982). The human oncogenes derived from bladder carcinomas have been cloned. They were found to be homologous to the oncogene from the Harvey sarcoma virus (Parada et al., 1982; Goldfarb et al., 1982; Santos et al., 1982).

In the following section I will summarize the evidence that cellular oncogenes become activated in cancer, and concentrate on the *myc* gene, one of the best studied examples.

The activation of the cellular *myc* gene was first shown in experiments involving avian leukosis virus (ALV). This virus, lacking an oncogene, induces B-cell lymphomas after a long latency. It turned out that the ALV genome is integrated in the tumor DNA near the cellular *myc*-gene. The integrated virus contains promoter sequences at its 3' end, which can direct transcription into flanking host sequences (Hayward et al., 1981). This induction of *myc* by nearby ALV genes is called promoter insertion. The viral promoter forms part of a larger sequence, which occurs at both ends of the integrated virus and resembles the insertion-like elements (IS) identified in prokaryotes (see Diggelmann, this volume). This suggests that integration of IS-like elements near an oncogene may trigger its expression. Indeed, Rechavi et al. (1982) recently found that one allele of the *mos* gene in a murine plasma-cytoma carried at the 5' end an insertion with direct and indirect repeats typical for IS-elements. It will be interesting to see if this observation is a general one. Evidence suggesting a possible role of the *myc* gene in human B-cell tumors has recently been presented. In man, the *myc* gen is located on chromosome 8. In Burkitt's lymphoma, as well as in other B-cell neoplasias, there is a typical translocation involving chromosomes 8 and 14, or, more rarely 8 and 22. Part of the long arm of 8 is translocated to chromosomes 14 (see Müller, this volume). Molecular cloning experiments show that in this translocation the *myc* gene, located on chromosome 8, becomes joined to the H-chain locus which is active in immunoglobulin-producing B-cells. In the more rare t (8; 22) translocation *myc* appears to become translocated to the lambda L-locus (Dalla-Favera et al., 1982; Taub et al., 1982; Nell et al., 1982). The translocation of the *myc* gene to the H-chain locus is also observed in plasmacytomas of mice which show a typical t (15; 12) translocation. In mice, *myc* is located on chromosomes 15 and the H-chain gene on chromosome 12 (Taub et al., 1982; Shen-Ong et al., 1982). In conclusion, the *myc* oncogene, known to cause experimental myelocytomatosis following MC29 virus infection is involved in a specific and virtually pathognomonic chromosome translocation in human and murine leukemias.

Activated oncogenes in human leukemias have also been detected using the NIH-3T3 transfection technique. Lane et al. (1981) described 5 oncogenes derived from human and murine T- and B-cell leukemias and lymphomas. Interestingly, different oncogenes were associated with pre-B, intermediate-B, mature-B, intermediate-T and mature-T cell tumors. It remains to be seen whether these genes are actually contributing to the malignant phenotype of these leukemias and lymphomas, or whether their activation occurs normally in differentiation and becomes "frozen" if the cell is immortalized by the transforming event.

In conclusion, the study of retroviruses and the development of suitable transfection techniques has led to the discovery of a fascinating family of genes, the oncogenes. There is hope that the elucidation of their function both in normal cells and experimental tumors will contribute to the understanding of some pathogenetic mechanisms underlying human cancer as well.

References

Aaronson, S.A., Todaro, G.J., and Scolnick, E.M. (1971) Induction of murine C-type viruses form clonal lines of virus-free BALB/3T3 cells. Science *174,* 157–159.

Aaronson, S.A., and Dunn, C.Y. (1974) Endogenous C-type viruses BALB/c cells: frequencies of spontaneous and chemical induction. J. Virol. *13,* 181–185.

Aaronson, S.A., and Stephenson, J.R. (1976) Endogenous type-C viruses of mammalian cells. Biochem. Biophys. Acta *458,* 323–354.

Aksamit, R.R., and Long, C.W. (1977) Induction of endogenous murine type C virus by an arginine analog: L-canavanine. Virology *78.* 567–570.

Dalla-Favera, R., Bregni, M., Erikson, J., Patterson, D., Gallo, R.C., and Croce, C.M. (1982) Human *c-myc onc* gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. Proc. Natl. Acad. Sci. *79,* 7824–7827.

Datta, S.K., and Schwartz, R.S. (1977) Mendelian segregation of loci controlling xenotropic virus production in NZB crosses. Virology *83,* 449–452.

Goldfarb, M., Shimizu, K., Perucho, M., and Wigler, M. (1982) Isolation and preliminary characterization of a human transforming gene from T24 bladder carcinoma cell. Nature *296,* 404–409.

Gross, L. (1951) "Spontaneous" leukemia developing in C3H mice following inoculation in infancy, with Ak-leukemic extracts, or Ak-embryos. Proc. Soc. Exp. Biol. Med. *76,* 27–32.

Groudine, M., Eisenman, R., and Weintraub, H. (1981) Chromalin structure of endogenous retroviral genomes and activation by an inhibitor of DNA methylation. Nature *292,* 311–317.

Hartley, J.W., Wolford, N.K., Old, L.J., and Rowe, W.P. (1977) A new class of murine leukemia virus associated with development of spontaneous lymphomas. Proc. Natl. Acad. Sci. *74,* 789–792.

Hayward, W.S., Neel, B.G., and Astrin, S.M. (1981) Activation of a cellular onc gene by promoter insertion in ALV-induced lymphoid leukosis. Nature *290,* 475–480.

Horak, I., Enjuaner, L., Lee, J.C., and Ihle, J.N. (1981) Resistence of cultures of normal T-cells to infection with murine C-type viruses. J. Virol, *37,* 483–487.

Klenner, D., Horak, I., Schimpl, A., and Wecker, E. (1982) Expression of endogenous retroviral glycoprotein 70 by antigen-activated cytotoxic and suppressor T lymphocytes of mice. Proc. Natl. Acad. Sci. USA *79,* 1250–1253.

Kozak, C.A., and Rowe, W.P. (1979) Genetic mapping of the ectropic murine leukemia virus-inducing locus of BALB/c mouse to chromosome 5. Science *204,* 69–71.

Kozak, C.A. and Rowe, W.P. (1980) Genetic mapping of xenotropic murine leukemia virus-inducing loci in five mouse strains. J. Exp. Med. *152,* 219–228.

Lane, M.-A., Sainten, A., and Cooper, G.M. (1981) Activation of related transforming genes in mouse and human mammary carcinomas. Proc. Natl. Acad. Sci. USA *78,* 5185–5189.

Lane, M.-A., Sainten, A., and Cooper, G.M. (1982) Stage-specific transforming genes of human and mouse B- and T-lymphocyte neoplasma. Cell *28,* 873–880.

Levy, J.A. (1973) Xenotropic viruses associated with NIH Swiss, NZB, and other mouse strains. Science *182,* 1151–1153.

Levy, J.A., Ihle, J.N., Oleszko, O., and Barnes, R.D. (1975) Virus-specific neutralization by a soluble non-immunoglobulin factor found naturally in normal mouse sera. Proc. Natl. Acad. Sci. USA *72,* 5071–5075.

Levy, J.A. (1978) Xenotropic type C-viruses. Curr. Top. Microbiol. Immunol. *79,* 109–213.

Lowy, D.R., Rowe, W.P., Teich, N., and Hartley, J.W. (1971) Murine leukemia virus: High frequency activation *in vitro* by 5-iododeoxyuridine and 5-bromodeoxyuridine. Science *174,* 155–156.

Moroni, Ch., and Schumann, G. (1975) Lipopolysaccharide induces C-type virus in short-term cultures of BALB/c spleen cells. Nature *254,* 60–61.

Moroni, Ch., and Schumann, G. (1978) Mitogen induction of murine C-type viruses. IV. Effects of lipoprotein *E. coli,* pokeweed mitogen and dextran sulphate. J. gen. Virol. *38,* 497–503.

Moroni, Ch., Stoye, J.P., DeLamarter, J.F., Erb, P., Jay, F., Jongstra, J., Martin, D., and Schumann, G. (1979) Normal B-cell activation involves endogenous retroviral antigen expression: Implications for leukemogenesis. Cold Spring Harbor Symp. Quant. Biol. *44,* 1205–1210.

Morse, H.C.III, Chused, T.M., Boehm-Truitt, M., Mathieson, B.J., Sharrow, S.O., and Hartley, J.W. (1979) Xen CSA: cell surface antigens related to the major glycoproteins (gp70) of xenotropic murine leukemia viruses. J. Immunol. *122,* 443–454.

Murray, M.J., Shilo, B.-Z., Shih, C., Cowing, D., Hsu, H.W., and Weinberg, R.A. (1981). Three different human tumor cell lines contain different oncogenes. Cell *25,* 355–361.

Neel, B.G., Ghanwar, S.C., Chaganti, R.S.K., and Hayward, S.W. (1982) Two human *c-onc* genes are located on the long arm of chromosome 8. Proc. Natl. Acad. Sci. USA *79,* 7842–7846.

Niwa, O., and Sugahara, T. (1981) 5-Azacytidine induction of mouse endogenous type C virus and suppression of DNA methylation. Proc. Natl. Acad. Sci. USA *78,* 6290–6294.

Parada, L.F., Tabin, C.J., Shih, C., and Weinberg, R.A. (1982) Human EJ bladder carcinoma oncogen is homologue of Harvey sarcoma virus *ras* gene. Nature *297,* 474–478.

Perucho, M., Goldfarb, M., Shimizu, K., Lama, C., Fogh, J., and Wigler, M. (1981) Human tumor-derived cell lines contain common and different transforming genes. Cell *27,* 467–476.

Phillips, S.M., Stephenson, J.R., and Aaronson, S.A. (1977) Genetic factors influencing mouse type-C RNA virus induction by naturally occurring B-cell mitogens. J. Immunol. *118,* 662–666.

Pulciani, S., Santos, E., Lauver, A.V., Long, L.K., Aaronson, S.A., and Barbacid, M. (1982) Oncogenes in solid human tumors. Nature *300,* 539–542.

Rascati, R.J., and Tennant, R.W. (1978) Induction of endogenous murine retrovirus by hydroxyurea and related compounds. Virology *87,* 208–211.

Rechavi, G., Givol, D., and Canaani, E. (1982) Activation of a cellular oncogene by DNA rearrangement: possible involvement of an IS-like element. Nature *300*, 607–611.

Rowe, W.P., Lloyd, M.W., and Hartley, J.W. (1980) The status of the association of MCF viruses with leukemogenesis. Cold Spring Harbor Symp. Quant. Biol. *44*, 1265–1268.

Santos, E., Tronick, S.R., Aaronson, S.A., Pulciani, S., and Barbacid, M. T24 human bladder carcinoma oncogene is an activated form of the normal human homologue of BALB- and Harvey-MSV transforming genes. Nature *298*, 343–347.

Schwartz, R.S., and Beldotti, L. (1965) Malignant lymphomas following allogenic disease = transition from an immunological to a neoplastic disorder. Science *149*, 1511–1514.

Shen-Ong, G.L.C., Keath, E.J., Piccoli, S.P., and Cole, M.D. (1982) Novel *myc* oncogene RNA from abortive immunoglobulin-gene recombination in mouse plasmacytomas. Cell *31*, 443–452.

Shih, C., Padhy, L.C., Murray, M., and Weinberg, R.A. (1981) Transforming genes of carcinomas and neuroblastomas introduced into mouse fibrolasts. Nature *290*, 261–264.

Stephenson, J.R., and Aaronson, S.A. (1972) A genetic locus for inducibility of C-type virus in BALB/c cells: the effect of a nonlinked regulatory gene on detection of virus after chemical activation. Proc. Natl. Acad. Sci. USA *69*, 2798–2801.

Stoye, J.P., and Moroni, Ch. (1983) Endogenous retrovirus expression in stimulated murine lymphocytes: identification of a new locus controlling mitogen induction of a defective virus. J. Exp. Med. (in press).

Taub, R., Kirsch, I., Morton, C., Lenoir, G., Swan, D., Tronick, S., Aaronson, S., and Leder, P. (1982). Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. Proc. Natl. Acad. Sci. USA *79*, 7837–7841.

Teich, N., Lowy, D.R., Hartley, J.W., and Rowe, W.P. (1973) Studies of mechanism of induction of infectious murine leukemia virus from AKR mose embryo cell lines by 5-iododeoxyuridine and 5-bromodeoxyuridine. Virology *51*, 163–173.

Tung, J.S., Vitetta, E.S., Fleissner, E., and Boyse, E.A. (1975) Biochemical evidence linking the G_{IX} thymocyte surface antigen to the gp69/71 envelope glycoprotein of murine leukemia virus. J. Exp. Med. *141*, 198–205.

Wecker, E., Schimpl, A., and Hünig, T. (1977) Expression of MuLV gp71-like antigen in normal mouse spleen cells induced by antigenic stimulation. Nature *269*, 598–600.

Wecker, E., and Horak, I. (1982) Current topics in immunology, *98*, 27–36.

Weiss, R., Teich, N., Varmus, H., Coffin, J. (Eds.) RNA Tumor Viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, 1982.

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Mouse mammary tumor virus: A model system for regulation of gene expression

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Zusammenfassung

Mouse mammary tumor virus (MMTV) kommt prizipiell in zwei Formen vor. Erstens als integrierte virale DNA (endogen vererbt), die in allen Zellen der Maus enthalten ist und zweitens als infektiöse Form, bei der sich die DNA nur im Kern von Brustdrüsenzellen integriert. Die erste Form verhält sich wie ein stummes Gen während die zweite Form aktiv ist, durch Glukocorticoide stimuliert wird und zum Mamma-Karzinom führt. Wir haben beide Typen von viralen Genen molekular geklont und durch Transfektion in verschiedene Zellen in Gewebekultur eingeführt. Wir konnten zeigen, dass sowohl die endogene DNA, wie die infektiöse DNA in transfektierten Zellen aktiv ist und dass die Expression beider Gene durch Glukocorticoide stimuliert wird. Wir konnten die DNA Sequenzen, die für die Hormonstimulierung nötig sind, in einem kleinen Fragment der viralen DNA lokalisieren. Bei der Sequenzanalyse dieses DNA-Stückes haben wir ein neues virales Gen entdeckt, das die Information für ein Protein von ca. 40000 Molekulargewicht enthält. Mit Hilfe eines Antikörpers suchen wir in verschiedenen Brustdrüsenzellen und- Tumoren nach diesem Protein, dessen Funktion noch nicht bekannt ist.

Introduction

The biology of the mouse mammary tumor virus (MMTV) has several features that make it particularly interesting. Its target cell specificity results in the appearance of mammary carcinomas in mouse strains with high mammary tumor incidence. There exist numerous strains of MMTV, which are transmitted either horizontally as infectious agents in the milk, or vertically, in form of integrated viral DNA (provirus) in the germ line. Infection of mice with milk-borne (exogenous) virus generally leads to mammary tumors early in life. Among the genetically transmitted (endogenous) variants, some have been associated with slowly growing mammary tumors late in life. Others seem to have no effect on tumor incidence (for review see Bentvelzen and Hilgers, 1980). Whether variations in the viral sequences, different genomic locations or differences in the target cells are responsible for the different patterns of expression was one of the questions asked in our studies.

Another peculiarity of MMTV is its hormonal regulation (reviewed by Varmus *et al.*, 1979). Both virus-induced mammary tumors and viral gene expression in tissue culture cells are regulated by glucocorticoid hormones. The increase in viral gene expression seems to be due to a direct action of the receptor-hormone complex. The experiments summarized below aimed at the localization of the DNA sequences responsible for the glucocorticoid response.

The mechanism by which MMTV causes mammary tumors is still unclear. The lack of a simple tissue culture transformation assay made it extremely difficult to tackle this problem. As large amounts of molecularly cloned MMTV DNA are now available for transfection studies new approaches to this problem can be envisaged.

Comparison of the biological activity of exogenous and endogenous MMTV DNA in transfected mouse and mink cells

Recently we molecularly cloned the unintegrated DNA of GR-MMTV (Buetti *et al.*, 1981) and an integrated endogenous provirus from AJ mice (Diggelmann *et al.*, 1982). Both types of proviruses have been transfected into mouse L-cells and their expres-

sion has been studied. The cloned exogenous provirus is efficiently expressed in transfected cells, as it is *in vivo* in infected mammary gland cells and in mammary tumors. Viral gene expression is also strongly stimulated by glucocorticoid hormones as it is *in vivo*, demonstrating that viral sequences are sufficient for hormone susceptibility (Buetti and Diggelmann, 1981). Endogenous proviruses which are not expressed *in vivo* and which do not respond to glucocorticoid stimulation (Dudley *et al.*, 1978) are capable of being normally expressed after cloning in bacteria and transfection into L-cells. These experiments (Hynes *et al.*, 1981; Diggelmann *et al.*, 1982) suggest that endogenous proviruses are potentially functional genes which are subject to a negative control in normal mouse cells. One of the important differences of endogenous proviruses *in vivo* and after transfection is their degree of methylaction. The L-cell endogenous, silent proviruses are highly methylated (Diggelmann *et al.*, 1982; Hynes *et al.*, 1981b). The transfection process in itself does not activate these proviruses and their methylation pattern does not change. By its replication in E. Coli the cloned MMTV DNA loses its specific methylation pattern and it remains undermethylated after transfection into mouse L-cells for as long as it has been examined (over one year). It is reasonable to assume that undermethylation is at least one condition for expression of MMTV DNA. These results ae summarized in table 1.

Table 1

	Endogenous provirus		Exogenous provirus	
	in vivo	after cloning and transfection	in vivo	after cloning and transfection
Integration of DNA	+	+	+	+
Viral RNA synthesis	—	+	+	+
Viral protein syntheis	—	+	+	+
Viral particles	—	not done	+	+
Glucocorticoid stimulation of transcription	—	+	+	+
Methylation of MMTV DNA	high	low	low	low
Oncogenicity of provirus	low	no results yet	high	no results yet

To exclude the possibility that the observed expression of viral products in transfected mouse cells resulted from recombination of the transfecting DNA with cellular sequences, or by activation of cellular MMTV copies, it was necessary to demonstrate the biological activity of these MMTV clones in cells which do not contain MMTV-related sequences. We therefore transfected the same DNA's into mink lung epithelial cells (CC164). Transformation by SV40 DNA was used as a selective marker to isolated transfected cells containing MMTV DNA. The results demonstrated that MMTV DNA of endogenous and exogenous origin was expressed in mink cells and viral gene expression was hormonally stimulated in both cases (Owen and Diggelmann, 1982). These experiments further confirm that the cloned endogenous and exogenous MMTV DNA's contain the complete viral information and that the sequences responding to glucocorticoid hormones are carried on the viral genome.

To localize the hormone responding sequences within the viral DNA we performed transfection studies with subgenomic DNA fragments. Figure 1A shows a schematic map of MMTV DNA and the fragments used for these experiments. The 1.45 kb Pst I fragment containing almost the complete region of the long terminal repeat (LTR) plus a few unique DNA sequences has been transfected into Ltk^- cells and we were able to demonstrate hormone stimulated synthesis of viral RNA (fig. 1B spots 4 and 5) (Fasel *et al.*, 1982) in these cells. This suggests that the hormone responsive sequence is located within the 1.45 kb Pst I fragment.

We also transfected Ltk^- cells with the 2.6 kb Sst I fragment which contains 103 bases upstream from the initiation site of viral RNA synthesis and reaches into the pol gene (see fig. 1A). One transfected cell clone obtained in this experiment synthesized considerable amounts of viral RNA, but dexamethasone had no effect on transcription (fig. 1B, spots 6, 7, 8). This result suggests that the hormone responsive sequences are located more than 70 nucleotides upstream from the

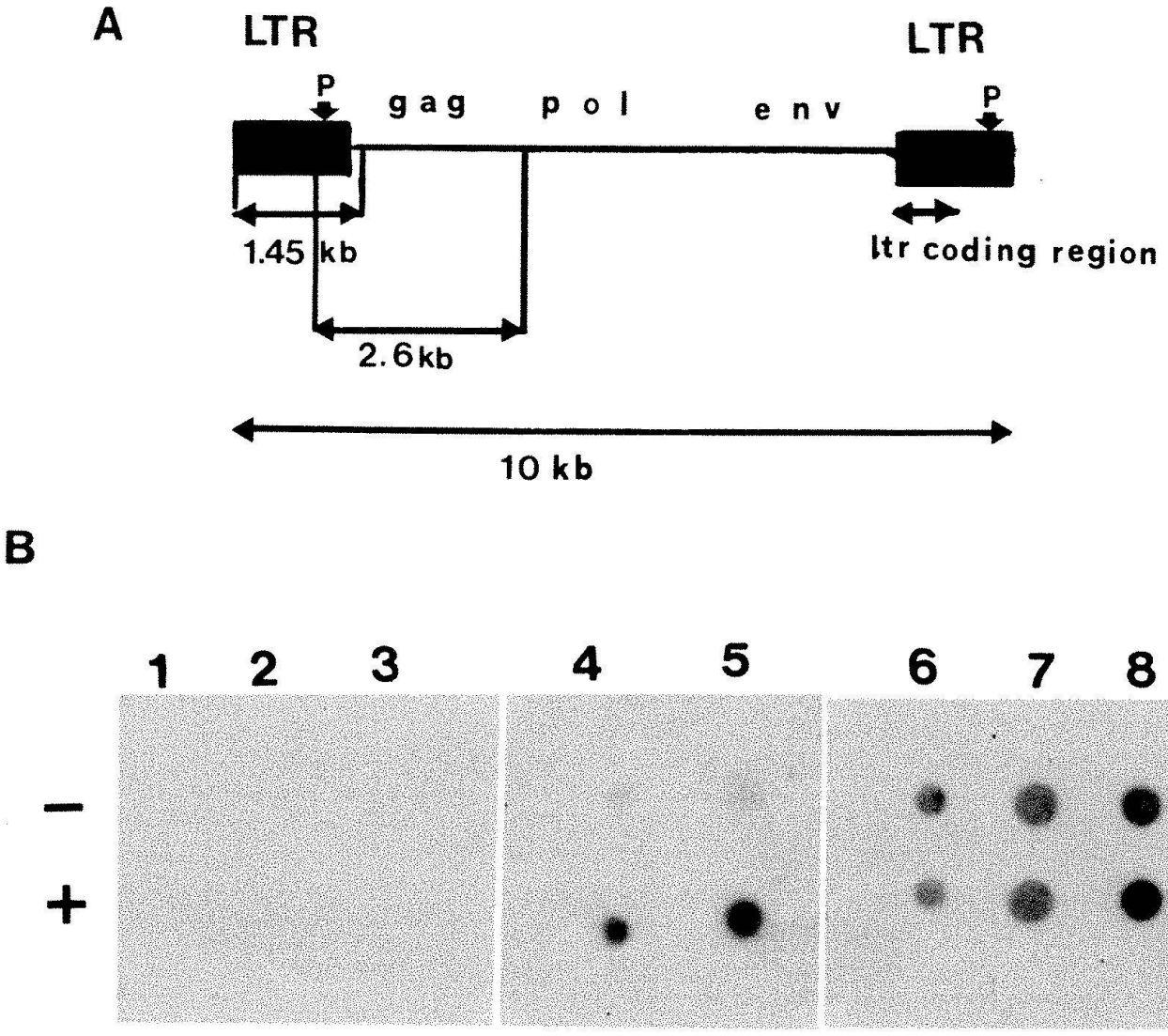

Fig. 1. A. Schematic representation of an MMTV genome. P = site of promotor for viral transcription. gag = gag-gene (major internal structural proteins). pol = polymerase gene (reverse transcriptase). env = envelope gene (glycoproteins). LTR = long terminal repeat. 1.45 kb fragment (Pst I restriction enzyme fragment). 2.6 kb fragment (Sst I restriction enzyme fragment).

B. Analysis of virus specific RNA synthesized in Ltk^- control cells (1, 2, 3) and Ltk^+ cells transfected with the 1.45 kb fragment (4, 5) or the 2.6 kb fragment (6, 7, 8). − total RNA from cells kept without hormone. + total RNA from cells kept overnight in 10^{-6} M dexamethasone 1, 4, 6 10 ug RNA spotted, 2, 5, 7 20 ug RNA spotted, 3, 8 30 ug RNA spotted. Filters were hybridized with ^{32}...-labelled MMTV DNA.

viral promotor (P in fig. 1A). Further transfection experiments using DNA fragments with deletions in the important region of -70 to -1100 upstream of the viral promotor are in progress.

Does MMTV have a transforming gene or does it cause mammary gland cell transformation by an alternative mechanism?

Retroviruses can be divided into two classes with respect to their mechanism of cell transformation. The first group (e.g. the classical Rous Sarcoma Virus) contains a transforming gene (viral *onc* gene). This gene is derived from a cellular gene (cellular onc gene) present in all animal cells. It is thought that these genes were picked up by passage of retroviruses in their host cells and by reverse transcription of the cellular onc gene mRNA (for review see Bishop, 1982). Viruses containing such an onc gene transform cells rapidly and cause tumors with high frequency and early in the life of the host. Viruses which do not contain an onc gene are capable of transforming cells by activation of a cellular onc gene (Neel *et al.*, 1981).

Viruses transforming cells by this mechanism cause tumors much more rarely and late in the life of the animal.

When we sequenced the long terminal repeat region of MMTV DNA we found a coding region of 960 basepairs potentially giving rise to a protein of 320 amino acids (36 K) (Fasel et al., 1982). Such a protein could be involved in mammary gland cell transformation. On the basis of the proposed amino acid sequence B. Gutte (University of Zürich) synthesized a peptide of 23 amino acids. This oligopeptide has been injected into rabbits in order to produce antibodies. We are now searching for the corresponding protein in normal and lactating mammary gland cells, in mammary tumors and in cells transfected with cloned MMTV DNA. In preliminary experiments using these antibodies we found a 50 K protein which is present at highest levels in lactating mammary gland cells, detectable in mammary tumor cells and transfected cells but not in normal mammary gland cells. This protein is larger than predicted by the sequencing data. This could be due to modifications of the protein; e.g. other transforming proteins are phosphoproteins. Experiments to characterize this 50 K protein in more detail are under way. Its function is for the moment still unknown. Much more work has still to be done to understand the mode of tumor formation by MMTV.

References

Bentvelzen, P., and Hilgers, J. (1980) Murine Mammary Tumor Virus: In Viral Oncology, G. Klein, ed. (New York: Raven Press) pp. 311–355.

Buetti, E., and Diggelmann, H. (1981) Cell *23*, 335–345.

Bishop, J.M. (1982) Scientific American *246*, 68–78.

Diggelmann, H., Vessaz, A.L., and Buetti, E. (1982) Virology *122*, 332–341.

Dudley, J.P., Butel, J.S., Socher, S.H., and Rosen, J.M. (1978) Virology *96*, 453–462.

Fasel, N., Pearson, K., Buetti, E., and Diggelmann, H. (1982) The EMBO Journal *1*, 3–7.

Hynes, N.E., Kennedy, N., Rahmsdorf, U., Groner, B. (1981a) Proc. Natl. Acad. Sci. USA *78*, 2038–2040.

Hynes, N.E., Rahmsdorf, U., Kennedy, N., Fabiani, L., Michalides, R., Nusse, R., and Groner, B. (1981b) Gene *16*, 307–317.

Neel, B.G., Hayward, W.S., Robinson, H.L., Fang, J., and Astrin, S.M. (1981) Cell *23*, 323–334.

Owen, D., and Diggelmann, H. (1983) J. Virol., in press.

Varmus, H.E., Ringold, G., and Yamamoto, K.R. (1979) In Monographs of Endocrinology. Glucocorticoid Hormone Action. J.D. Baxter and G.G. Rousseau, eds. (New York, Spinger) pp. 253–278.

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Clinical and biological significance of chromosome aberrations in leukemia and lymphoma*

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Summary

Clonal chromosome aberrations have been found in several malignancies, especially in leukemia and lymphoma. Their exact characterization has diagnostic, therapeutic and prognostic significance. Consistently occurring translocations have been found in several subsets of acute non-lymphocytic leukemia and in chronic myeloid leukemia as well as in lymphoid neoplasms.

These aberrations tend to affect only certain chromosomes. Specific segments along these chromosomes are preferentially involved in structural aberrations. This may imply that these chromosomes carry genetic material of special importance for malignancies. Cancer genes known as "oncogenes" have been identified within affected chromosomal segments. By pooling the data about the chromosome aberrations and malignancy it becomes apparent that there might be at least two main genetic mechanisms operating in human neoplasia. In the majority of the reported solid tumors as well as in some leukemias and lymphomas there is a loss of specific chromosome material implying that in these malignancies there might be a critical loss of genetic information. In most leukemias and lymphomas a reciprocal translocation with precise breakpoints occurs; in this case it is possible that a cancer gene (oncogene) becomes activated as a result of the genetic rearrangement. Therefore, one common pathway to cancer may involve the direct activation of oncogenes; but there is also another pathway involving a loss of activity of genes whose function might be the induction of final differentiation. It has been suggested that the oncogenes are the driving factors of proliferation and that differentiation is controlled by a different group of genes which – in effect – act as anti-oncogenes (Knudson, 1982).

Zusammenfassung

Klonale Chromosomenaberrationen wurden in verschiedenen malignen Neubildungen, vor allem in Leukämien und Lymphomen, nachgewiesen. Ihre exakte Charakterisierung hat diagnostische, therapeutische und prognostische Bedeutung. Spezifische Chromosomentranslokationen wurden bei verschiedenen Formen der akuten nichtlymphatischen Leukämie, der chronisch myeloischen Leukämie, aber auch bei bösartigen Neubildungen des lymphatischen Gewebes gefunden.

Von solchen Aberrationen werden bestimmte Chromosomen und oft sogar nur Chromosomensegmente betroffen. Dies ist ein Hinweis dafür, dass dort Gene lokalisiert sein müssen, die eine entscheidende Rolle bei der Entstehung eines bösartigen Tumors spielen. Tumorgene, die Onkogene genannt werden, kommen in solchen Regionen vor. Die Vermehrung oder auch Aktivierung von Onkogenen führt wahrscheinlich zu unkontrollierter Proliferation. Zahlreiche Neoplasien sind aber durch einen Verlust von Chromosomenmaterial gekennzeichnet. Demzufolge muss es noch Erbfaktoren geben, deren Verlust im Zusammenhang mit der Tumorgenese von Bedeutung ist. Es könnte sich dabei um Erbfaktoren handeln, die für die endgültige Differenzierung der Zellen verantwortlich sind. Knudson (1982) hat die einfache Hypothese aufgestellt, dass Tumoren aus einem Missverhältnis der Aktivität der Onkogene und solcher Differenzierungsgene resultieren, die er Anti-Onkogene nennt.

*This paper is dedicated to Professor Dr. G. Stalder, Director of the University Children's Hospital, Basel, in honour of his 60th birthday.

Our own investigations on chromosomes in malignancies were supported by the Swiss Cancer League.

Introduction

An association between chromosome aberrations and neoplastic transformation has long been mooted (Bovery, 1914) since chromosomal abnormalities have been found in cells of a variety of malignancies. With the new banding techniques cancer cytogenetics has become a rapidly expanding branch of everyday oncology. Consistently occurring clonal chromosome aberrations have been found to be associated with specific types of leukemia and lymphoma (Rowley, 1980). Although such findings do not directly prove the hypothesis that chromosomal changes represent a major step in carcinogenesis, a remarkable concordance between the chromosomal location of cellular oncogenes and the chromosomal segments involved in such aberrations is becoming apparent. In this review the clinical and biological significance of chromosomal rearrangements in leukemic cells will be discussed.

1. Clinical significance

Several major types of chromosome abnormalities have been identified in human leukemias and lymphomas. They have been found to exhibit a wide range of defects, i.e. a gain or a loss of entire chromosomes or parts of chromosomes and also structural rearrangements such as reciprocal translocations. Certain chromosomes are preferentially affected. Their identification refines the classification of the malignancies.

1.1. Chronic myeloid leukemia (CML)

The Philadelphia (Ph^1)-chromosome in CML was described in 1960 by Nowell and Hungerford. On the basis of banded karyotypes, this aberration was first interpreted as a 22q-deletion (Caspersson et al, 1970) and was subsequently found to involve primarily a translocation between a chromosome No. 9 and a No. 22: t(9;22)(q34;q11) (Rowley, 1973). Approximately 90% of all patients suffering from CML show the presence of the Ph^1-chromosome.

Patients with the Ph^1-negative form tend to be older than those with the Ph^1-positive form. They are predominantly male and have a smaller leukocyte increase and more pronounced thrombocytopenia. As a general rule the Ph^1-negative patients show a poor response to therapy, so that survival time is shorter (Ezdlinli et al, 1970). After a period of three years 84% of 23 of our own patients with a Ph^1-positive CML were still alive, compared with only 60% of 8 patients with the Ph^1-negative form (unpublished results).

At the time of blast crisis about 75% of all patients develop additional chromosome aberrations, superimposed on the karyotype with only the Ph^1-chromosome (Mitelman and Levan, 1981). The predominant aberrations are a gain of a second Ph^1-chromosome or of one or more chromosomes No. 8, or the formation of an isochromosome No. 17. Altogether, at least one of three changes occurs alone or in combination in more than 80% of the karyotypically abnormal CML-patients.

Table 1. Consistent structural chromosome aberrations in acute non-lymphocytic leukemia

Type of ANLL	Translocation/Inversion	Clinical, cytological and other findings
Acute myeloblastic leukemia, AML M2	t(8:21) (q22:q22)	Low onset age, good response to treatment, 2/3 of myeloblasts with Auer rods, low alkaline phosphatase
Acute promyelocytic leukemia, APL M3 and variant M3	t(15:17) (q22:21)	Hypergranular promyelocytes, tendency to intravascular coagulation, poor morphology of the chromosomes
Acute monocytic leukemia AMoL M5 (auch M4)	11q- (q23-24) (q13-14) translocation variable	Most frequently in children with type a
Acute myelocytic leukemia M1	t(9:22) (q34:q11) Philadelphia chromosome	Poor response to therapy, short survival time
Acute myelomonocytic leukemia AMMoL M4	inv(16) and del (16)(q22)	Increased number of eosinophils, good prognosis

In a 17-year old female patient with a Ph[1]-positive CML a deletion of the long arm of one chromosome No. 11 was found in an acceleration phase of the disease which did not turn into a typical blast crisis, but led to death.

1.2. Acute non-lymphocytic leukemia (ANLL)

Abnormal karyotypes have been reported in approximately 50% of all patients with ANLL (Sandberg, 1980). Analysis of metaphase plates alone showed clonal aberrations in 25 of 55 of our own patients (unpublished results). However, the incidence of chromosomal aberrations may be significantly greater when new techniques for obtaining prometaphase chromosomes are applied (Yunis et al. 1981).

The specific chromosome aberrations which are most commonly found in ANLL have been reviewed extensively (Mitelman and Levan, 1981; Sandberg, 1980). Therefore, only the translocations will be presented that are specifically associated with particular types of ANLL.

There seem to be at least five clinical subtypes of ANLL that can be identified by the type of chromosome rearrangement found (see tab. 1).

One group has a translocation involving the long arm of chromosomes No. 8 and 21 and represent about 10 to 15% of all ANLL patients with chromosomal aberrations (Kamada et al, 1968; Rowley, 1973). They have the hematological picture of an acute myelogenous leukemia M2 and are recognized from the low onset age of the leukemic process. The myeloblasts often have Auer rods and a low alkaline phosphatase activity. Patients with this disorder respond well to therapy and have a relatively good prognosis. Experienced cytologists recognise this type as a special entity because of the specific morphologic features of the leukemic cells.

A second group is characterized by a structural rearrangement between chromosomes No. 15 and 17. This translocation is unique to acute promyelocytic leukemia (M3 and M3 variant) (Rowley et al. 1977).

The third group shows a typical Philadelphia chromosome; the patients suffer form AML with a poorer response to therapy. It is not known why the same translocation, occurring probably in similar stem cells, leads to different hematological pictures, not only CML and AML but also ALL.

In patients with acute myelomonocytic leukemia (M5) and also acute promyelocytic leukemia (M4) a translocation is observed to involve the long arm of chromosome No. 11 (Berger et al. 1980).

Finally, an inversion (Inv 16) is found in patients with acute monocytic leukemia (M4).

All these abnormalities occur preferentially in children and younger adults, indicating that the aetiological factors responsible for these types of leukemia differ from those associated with leukemia in older adults. Instead of having a specific translocation the latter show a variety of numerical and also structural defects, especially of chromosomes No. 5, 7, or 8.

Non-random chromosomal changes have been observed in the marrow cells of patients who have acute non-lymphocytic leukemia secondary to treatment with irradiation or cytotoxic drugs. These aberrations consist of a loss of the entire chromosome or part of the long arm of chromosomes No. 5 and/or 7 (Colomb et al. 1982). Patients who have been occupationally exposed to some potential mutagenic agents (petroleum products, chemical solvents or pesticides) were more likely to have chromosomally abnormal leukemic cells than non-exposed persons. The incidence of certain chromosome changes, such as of No. 5, 7 or 8 was much higher in the exposed than in the unexposed population (Mitelman et al. 1978).

Several of these chromosome aberrations show an uneven geographic distribution. Such geographic heterogeneity may be taken to indicate heterogeneity in the distribution of aetiologic agents.

Table 2. Median survival time and cytogenetic pattern in 241 patients with ANLL*.

Cytogenetic pattern	No. of patients	Median survival in months	Alive after 1 year No.	%
Normal	102	6	21	21
Normal and abnormal	80	5	17	21
Abnormal	59	4	3	5

* Results based on an analysis by the First Internal Workshop on Chromosomes in Leukemia (1978).

With a successful antileukemic therapy patients having cells with normal karyotypes are capable of repopulating their marrow with these elements when the leukemic ones disappear.
In addition chromosomal aberrations present in leukemic cells can be used to monitor a specific leukemic cell clone during the whole course of the disease. This is of considerable importance in phases such as clinical remission or relapse. In our hospital we profited very much from the information gained from cytogenetic analysis in different stages of the disease (Müller and Stalder, 1976). New sub-clones, carrying new chromosomal abnormalities, often acquire new phenotypic properties and are responsible for minor changes in the course of the disease.
Secondary chromosome aberrations may arise in malignancies through disturbance of mitosis in rapidly dividing cells. Also such secondary changes that happen to enhance the effect of the primary ones have a selective value and thus will accumulate in the cell population that means in subclones.

1.3 Lymphoid neoplasms
The karyotypic aberrations in many lymphoid neoplasms appear multiform with no evident consistent pattern. However, the different specific chromosome translocations have been identified in ALL: t(9;22), t(4;11), t(8;14) (Sandberg, 1980). These are the most common structural rearrangements as shown in table 3. As in ANLL the presence of aneuploid clones is a significant negative prognostic factor. The importance of diagnosing Ph^1-positive ALL both in children and in adults is now universally recognized. Most cases of lymphoma and B-cell ALL have a 14q-abnormality, the donor chromosome most frequently being No. 2, 8, 11, 22 or the other No. 14. The prognostic importance of the specific donor chromosome is not clear, but may correlate with specific immunoglobulin light or heavy chains found on the malignant cell surfaces (Croce et al. 1979; Erikson et al. 1981; McBride et al. 1982; Kirsch et al. 1982).

Table 3. Consistent chromosome abnormalities in lymphoid neoplasms

Chronic lymphocytic leukemia (B cells)	14q+ +12
Plasma cell leukemia (multiple myeloma)	14q+
ALL (B-cells)	14q+ t(8:14) t(8:22) t(11:14) t(4:11)
Various lymphomas	
a) endemic and non-endemic Burkitt's	14q+ t(8:14) t(2:8) t(8:22)
b) non-Hodgkin's, non-Burkitt's	14q+
c) malignant (follicular) lymphoma	t(14:18)

2. Biological significance

The genetic alteration responsible for the initiation of a malignancy probably does not take place at the chromosomal level but at the gene or DNA level. The origin and the role of the chromosome aberrations in this process are still matters of scientific debate. However, the facts that consistent chromosomal abnormalities occur in a high percentage of patients with certain types of cancer and that the chromosomal abnormalities affect only certain chromosomes or even chromosomal segments contradict the opinion that they represent only a symptom or an incidental phenomenon of the disease and have nothing to do with its initiation or progression.
When considering the genetic effects of chromosome breakage and rearrangement, it is important to bear in mind that despite the improved resolution achieved by new banding techniques human chromosome bands are far removed from single genes.
The human haploid genome contains some 3 x 10^9 nucleotide pairs. An average sized metaphase band therefore contains around 10^7 nucleotide pairs. This amount of DNA would be enough to code some 5000 different mRNA transcripts, each originally two kilobases long. The increased resolution obtained by investigating prometaphases represents a significant advance, but if there are some 40000 or so genes in the genome, then these 800 bands will relate to 50 or so units of information (Evans, 1982). Cancer genes, which are known as oncogenes (onc), are one of the "hottest" areas of current cancer re-

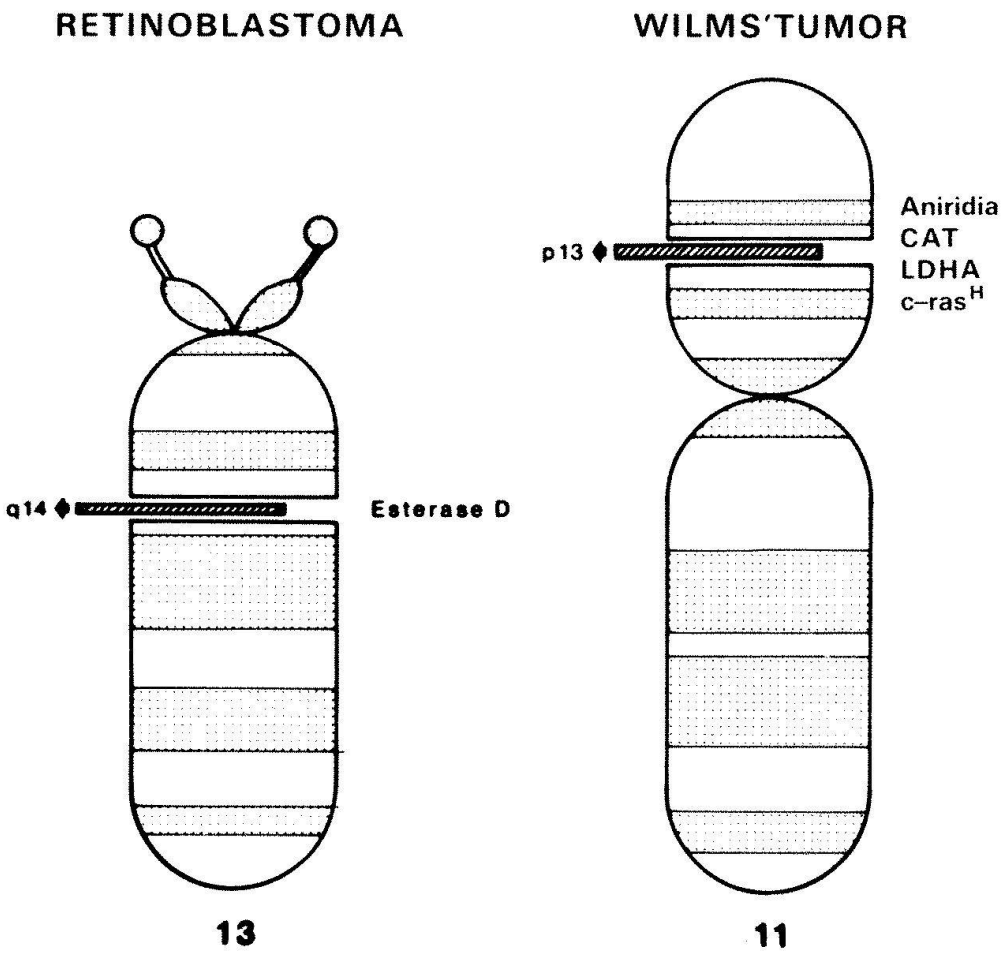

Fig. 1. Schematic representation of chromosome Nos. 11 and 13. Chromosomal assignment of the lost or inactivated genes for retinoblastoma and Wilms'tumor as well as of linked loci (see also: McKusick, 1983).

search. The 20 or so viral oncogenes have normal cellular counterparts. Sixteen such oncogenes have been identified within the last few months and the location of nine of them on the chromosomes has been identified (Bristol-Myers-Symposium, Chicago, October 1982). One of these oncogenes, known as "myc" is associated with an abnormal chromosome translocation that is found in almost all cases of Burkitt's lymphoma (Manolova et al, 1979). The end of chromosome No. 8 which contains the myc oncogene is translocated to the long arm of one No. 14 chromosome where the immunoglobulin heavy chain gene cluster is located. It is now assumed that this relocation of the myc oncogene affects its expression by the neighbouring active immunoglobuline genes (Croce et al, 1983). Loss of the normal regulatory control of the c-myc oncogene may cause malignancy. The specific association of certain translocations with particular leukemia and lymphoma suggests that some alterations in gene function give a proliferative advantage to specific groups of cells such as the B cells in the case of the myc oncogene.

In malignant cells one can observe cytogenetic phenomena which rarely occur in normal cells such as double minute chromosomes or homogeneously staining regions. There are indications that both phenomena correlate with gene amplification. The functional significance of these chromosomal structures in malignant cells is at present unknown. Further characterization of the amplified sequences is needed. It could be that some of these represent amplified oncogenes.

The study of hereditary cancers in man indicates the existence of another class of genes, not yet defined in precise terms (Knudson, 1982). These genes are transmitted in a dominant fashion and predispose to specific cancers. Classical examples are hereditary retinoblastoma or Wilms' tumor (see fig. 1). These genes must differ from oncogenes because there is evidence that in

some instances the gene site is deleted. It is therefore the loss of gene activity that is important.

The retinoblastoma exists in both heritable and non-heritable forms. A small percentage shows a specific deletion of chromosome No. 13 (13q;14) in all somatic cells (Franke and Kung, 1976). The remaining hereditary cases, according to studies of linkage with the gene for esterase D, involve the same site on chromosome No. 13, although there is no visible chromosomal change. Since esterase D and the retinoblastoma genes are closely linked and since it is known that esterase D can be expressed in tumors, there should be cases in which there is no esterase D activity in the tumor. In one case examined for this possibility the karyotype of the individual was normal (Benedict et al. 1982). However, esterase D activity in somatic cells was 50% of normal suggesting that there was a submicroscopic deletion of the 13q;14 site. In the tumor there was only one chromosome No. 13. Apparently the normal chromosome No. 13 was missing, because there was no measurable esterase activity in the tumor.

Pooling the data on chromosomal aberrations in leukemias and other malignancies the present information about oncogenes and genes which may act in hereditary tumors reveal at least two main genetic mechanisms operating in human neoplasms. In most leukemias and lymphomas a reciprocal translocation with precise breakpoints occurs; in these instances it is possible that a cancer gene becomes activated as a result of genetic rearrangement. Therefore it may be that one common pathway to cancer involves the direct activation of an oncogene while there is also an indirect pathway involving the loss of gene activity. This is indicated by the fact that in some leukemias and lymphomas and especially in the majority of the reported solid tumors, there is a loss of specific chromosomal segments, implying a critical loss of genetic information in these malignancies. The function of the lost genes may be to induce final differentiation. Knudson (1982) suggests that cell proliferation may be regulated by oncogenes and that differentiation may be controlled by a second set of genes which in effect are anti-oncogenes.

When compiling facts in a short sumary we always run the risk of oversimplification. However, it is felt that in such a complex field as oncology, simple hypotheses are needed which can be tested directly in order to make the necessary progress. There is no doubt that cytogenetic studies will become more and more useful in the clinical management of patients and that they may provide clues to the etiology of the disease and to the type of genes which are involved in malignancy.

Addendum

Since the article was first written in 1982 the recent application of DNA technology to tumor cytogenetics has provided a new insight into the nature of the chromosomal aberrations in leukemia and lymphoma. In the case of CML the oncogene c-abl is transferred from chromosome 9 to chromosome 22. Surprisingly, at the DNA level the breakpoints on the chromosomes vary considerably between individuals [Groffen et al. Cell *36*, 93 (1984)]. The recombinant DNA techniques also permit the detection of somatic events leading to retinoblastoma and Wilms' tumor. Cavernee and colleagues (Nature *305*, 139, 1983) have shown that at least in some tumors the malignant cells became homozygous for the chromosome 13 carrying the "retinoblastoma gene". Findings with Wilms'tumor also indicate that chromosomal mechanisms generating homozygosity may be important for tumor formation [Orkin at al. Nature *309*, 172 (1984)].

References

Benedict, W.F., Murphree, A.L., Banerjee, A.: Chromosomal changes in unilateral or bilateral retinoblastoma: Important differences and implications to etiology. Proc. Seventy-Third Annual Meeting of the American Association for Cancer Research (Abstract) 23.40 (1982).

Berger, R., Bernheim, A., Weh, H.: Cytogenetic studies on acute monocytic leukemia. Leukemia Res *4*, 119–127 (1980).

Biedler, J.L., Spengler, B.A.: Metaphase chromosome anomaly: Association with drug resistance and cell specific products. Science *191*, 185–187 (1976).

Boveri, T.: Zur Frage der Entstehung maligner Tumoren. Gustav Fischer, Jena (1914).

Caspersson, T., Gahrton, G., Linsten, J.: Identification of the Philadelphia chromosome as a number 22 by quinacrine mustard fluorescence analysis. Exp. Cell. Res. *63*, 238–244 (1970).

Croce, C.M., Shander, M., Martinis, J., Cicurel, L., D'Ancona, C.L., Dolby, T.W., Koprowski, H.: Chromosomal location of the genes for human immunoglobulin heavy chains. Proc. Natl. Acad. Sci. USA *76,* 3416–3419 (1979).

Croce, C.M., Thierfelder, W., Erikson, J. et al.: Transcriptional activation of an unrearranged and untranslocated c-myc oncogene by translocation of a C-lambda locus in Burkitt lymphoma cells. Proc. Natl. Acad. Sci. USA *80,* 6922–6926 (1983).

Erikson, J., Martinis, J., Croce, C.M.: Assignment of the genes for human λ immunoglobulin chains to chromosome 22. Nature (London) *294,* 173–175 (1981).

Evans, H.J.: Cytogenetics of heritability in cancer. In: Host factors in human carcinogenesis. (Bartsch, H., and Armstrong, B. eds) IARC Scientific Publications No. *39,* Lyon pp. 35–56 (1982).

Ezdinli, E.Z., Sokal, J.E., Crosswhite, L., Sandberg, A.A.: Philadelphia-chromosome-positive and -negative chronic myelocytic leukemia. Ann. Int. Med. *72,* 175–182 (1970).

Francke, U.: Retinoblastoma and chromosome 13. Cytogenet. Cell Genet. *16,* 131–134 (1976).

Golomb, H.M., Alimena, G., Rowley, J.D.: Correlation of occupation and karyotype in adults with acute non-lymphocytic leukemia. Blood *60,* 404–411 (1982).

Kamada, N., Okada, K., Ho, T., Nakatsui, T., Uchino, H.: Chromosomes 21–22 and neutrophil alkaline phosphatase in leukemia. Lancet *1,* 364 (1968).

Kirsch, I.R., Morton C.C., Nakahara, K., et al: Human immunoglobulin heavy chain genes map to a region of translocations in malignant B lymphocytes. Science *216,* 301–303 (1982).

Knudson Jr, A.G.: Contribution and mechanisms of genetic predisposition to cancer: hereditary cancers and anti-oncogens. Proceedings of the 13th International Cancer Congress 1982, Seattle, Washington (in press).

Lenoir G., Preud'homme J.L., Bernheim, A., et al: Correlation entre translocation variante et expression des chaînes légères d'immunoglobulines dans les lymphomes et leucémies de type Burkitt. CR. Acad. Sci. Paris *293,* 427–429 (1981).

McBride O.W., Hieter, P.A., Hollis, G.F., et al: Chromosomal location of human kappa and lambda immunoglobulin light chain constant region genes. J. Exp. Med. *155,* 1480–1490 (1982).

McKusick, V.: Mendelian Inheritance in man. Catalogues of autosomal dominant, autosomal recessive and X-linked phenotypes. The Johns Hopkins University Press, Baltimore, 6th edition (in press).

Manolova, Y., Monolov, G., Kieler, J.: Genesis of the 14q+ marker in Burkitt's lymphoma. Hereditas *90,* 5–10 (1979).

Mitelman, F., Brandt, L., Nilsson, P.G.: Relation among occupational exposure to potential mutagenic/carcinogenic agents, clinical findings and bone marrow chromosomes in acute non-lymphocytic leukemia. Blood *52,* 1229–1237 (1978).

Mitelman, F., Levan, G.: Clustering of aberrations to specific chromosomes in human neoplasms. IV. A survey of 1,871 cases. Hereditas *95,* 79–139 (1981).

Müller, Hj., Stalder, G.R.: Chromosomes and human neoplasms. Achievements using new staining techniques. Eur. J. Pediatr. *123,* 1–13 (1976).

Nowell, P.C., Hungerford, D.A.: A minute chromosome in human chronic granulocytic leukemia. Science *132,* 1497 (1960).

Riccardi, V.M., Sujansky, E., Smith, A.C., Francke, U.: Chromosomal imbalance in the aniridia-Wilms' tumour association: 11p interstitial deletion. Pediatrics *61,* 604–610 (1978).

Rowley, J.D.: Identification of a translocation with quinacrine fluorescence in a patient with acute leukemia. Ann. Genet. *16,* 109–112 (1973).

Rowley, J.D.: A new consistent chromosomal abnormality in chronic myelogenous leukemia. Nature *243,* 290–293 (1973).

Rowley, J.D.: Population cytogenetics of leukemia: In: Population cytogenetics (Porter, I.H., Hook, F.B. eds) New York, Academic Press, pp. 189–216 (1977).

Rowley, J.D.: Chromosome changes in acute leukemia Br. J. Haematol. *44,* 339–346 (1980).

Sandberg, A.A.: The chromosomes in human cancer and leukemia. New York, Elsevier-North-Holland (1980).

Yunis, J.J., Bloomfield, C.D., Ensrud, B.S.: All patients with acute non-lymphocytic leukemia may have a chromosomal defect. N. Engl. J. Med. *305,* 135–139 (1981).

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Growth control in cell-cycle mutants of animal cells*

Richard Schindler, Arthur Zimmermann, Jean-Claude Schaer and Jacques Schneider

Summary

In neoplastic cells, regulation of the cell-division cycle is defective, resulting in impaired control of entry of cells into a state of proliferative quiescence and/or of exit from the quiescent state into the division cycle. Conditional cell-cycle mutants represent attractive model systems for studies of mechanisms of regulation underlying the ordered progression in the cell cycle as well as entry into and exit from a state of proliferative quiescence. In this communication, the selection of a series of heat-sensitive (arrested at 39.5° C, exponentially multiplying at 33° C) and cold-sensitive (arrested at 33° C, exponentially multiplying at 39.5° C) cell-cycle mutants and some of their characteristics are described. At the respective nonpermissive temperature, the heat-sensitive as well as the cold-sensitive mutants enter into a state of reversible proliferative quiescence with a cellular DNA content identical to that of cells in G_1 phase. When mutant cells are fused to «wild-type» cells, the phenotype of arrested heat-sensitive cells is expressed in a recessive manner, while cold-sensitive cells behave dominantly under these conditions. Furthermore, the cold-sensitive mutant cells differ from the heat-sensitive cells by their capacity to undergo cellular differentiation with the formation of mast-cell granules when incubated at the nonpermissive temperature. Thus, the state of proliferative quiescence induced in heat-sensitive cells is qualitatively different from that of cold-sensitive cells, and the latter may be used as models in analyzing intracellular processes underlying entry of cells into a state of proliferative quiescence coupled with morphological cell differentiation.

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Zusammenfassung

Neoplasien weisen eine Störung in der Regulation des Zellteilungszyklus auf, indem die Kontrolle des Eintritts von Zellen in einen Zustand der Proliferationsruhe bzw. des Austritts aus der Proliferationsruhe in den Teilungszyklus bei Krebszellen defekt ist. Als vielversprechendes Modellsystem in der Analyse von Regulationsmechanismen, die dem Durchlaufen des Zellteilungszyklus sowie dem Erreichen und Verlassen der Proliferationsruhe zugrunde liegen, haben sich konditionelle Zellzyklus-Mutanten erwiesen. Die Selektion und die Eigenschaften einer Serie hitzeempfindlicher (Arretierung bei 39,5° C, exponentielle Zellvermehrung bei 33° C) und kälteempfindlicher (Arretierung bei 33° C, exponentielle Zellvermehrung bei 39,5° C) Zellzyklus-Mutanten eines Mäuse-Mastozytoms werden beschrieben. Bei der nicht-permissiven Temperatur treten sowohl die hitze- als auch die kälteempfindlichen Mutanten in einen Zustand reversibler Proliferationsruhe mit einem DNS-Gehalt entsprechend der G_1-Phase ein. Bei Fusion mit «Wild-Typ»-Zellen verhielten sich die arretierten hitzeempfindlichen Zellen phänotypisch rezessiv, arretierte kälteempfindliche Zellen dagegen dominant. Ein weiterer Unterschied zwischen den hitze- und kälteempfindlichen Zellzyklus-Mutanten besteht darin, dass sich bei der betreffenden nicht-permissiven Temperatur an den kälteempfindlichen Zellen eine Zelldifferenzierung unter Bildung von Mastzell-Granula nachweisen liess, während an den hitzeempfindlichen Zellen kein derartiger Differenzierungsprozess zu erkennen war. Die in den hitzeempfindlichen Zellzyklus-Mutanten induzierte Proliferationsruhe ist somit qualitativ verschieden von derjenigen in den kälteempfindlichen Mutanten, und letztere bieten sich als Modell für die Analyse der

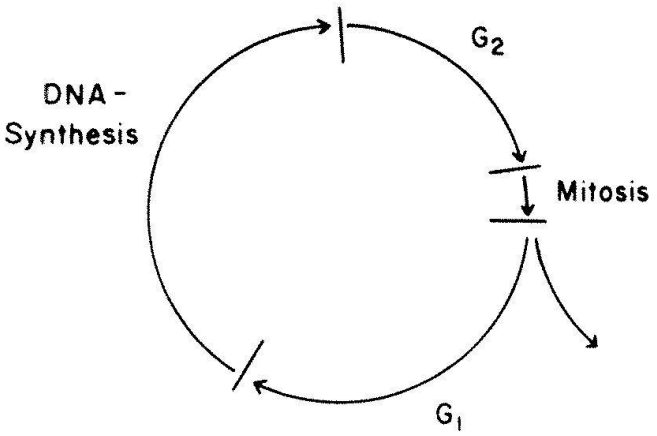

Fig. 1. The cell division cycle of animal cells.

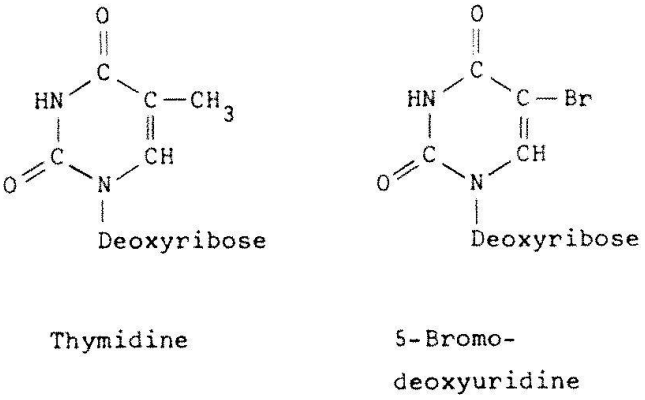

Fig. 2. Chemical structure of the DNA precursor, thymidine, and of its analog, 5-bromo-deoxyuridine.

intrazellulären Prozesse beim Eintritt normaler Zellen in die Proliferationsruhe unter gleichzeitiger Zelldifferenzierung an.

In the cell division cycle of mammalian and other animal cells, i.e. between two successive cell divisions, four different phases can be distinguished (fig. 1): G_1 phase; S phase in which the cellular genome in the nucleus is replicated; G_2 phase; and mitosis. Cells in mitosis are easily identified by their morphology, e.g. by condensation of nuclear chromatin into a specific number of chromosomes, while cells in S phase may be recognized autoradiographically by their capacity to incorporate ^{3}H-labeled thymidine into DNA. Cells in G_1 phase and those in G_2 phase may be distinguished by cytofluorometric determination of their DNA content (Tobey et al., 1975).

When animal cells are cultured in vitro under appropriate culture conditions, they may multiply exponentially over prolonged periods of time, resulting in an essentially constant duration of successive cell division cycles of usually below 30 hours. On the other hand, in the intact organism and under growth-limiting culture conditions, many cells have stopped their progression in the cell cycle between mitosis and S phase and are in a state of proliferative quiescence which may last many months, or even indefinitely. In this context, neoplasia may be defined as a defect in the control of entry into and/or exit from such a state of proliferative quiescence.

In the investigation of individual cellular functions responsible for ordered progression in the cell cycle and for entry into and exit from proliferative quiescence, conditional cell-cycle mutants of animal cells are considered to be promising model systems (Prescott, 1976; Baserga, 1978; Basilico, 1978; Simchen, 1978; Siminovitch et al., 1978). Cell-cycle mutants are operationally defined as mutants that are arrested, under the nonpermissive condition, in a specific phase of the cell cycle. The mutants described so far are heat-sensitive or cold-sensitive, and most of them are arrested in G_1 phase (i.e. between mitosis and S phase) at the nonpermissive temperature.

The method of selecting conditional cell-cycle mutants that has been applied most frequently is based on the use of 5-bromodeoxyuridine (BrdUrd) which is an analog of the normal DNA precursor substance, thymidine (dThd) (fig. 2). If 5-bromodeoxyuridine is present in the culture medium, it is efficiently incorporated by cells in S phase into their DNA. Cells containing 5-bromodeoxyuridine in place of thymidine in their DNA differ from cells containing normal DNA by their high sensitivity to light in the wave-length region around 400 nm. Cells with 5-bromodeoxyuridine-containing DNA may thus be killed selectively by irradiation with 400 nm light (Puck et al., 1967). The principle of selection based on the use of 5-bromodeoxyuridine and applied for isolation of heat-sensitive cell-cycle mutants is depicted in figure 3.

In our studies, cultured cells of a murine mast-cell tumor termed P-815 mastocytoma (Dunn et al., 1957; Schindler et al., 1959; Green et al., 1960) were used as the parent population for selection of both heat-sensi-

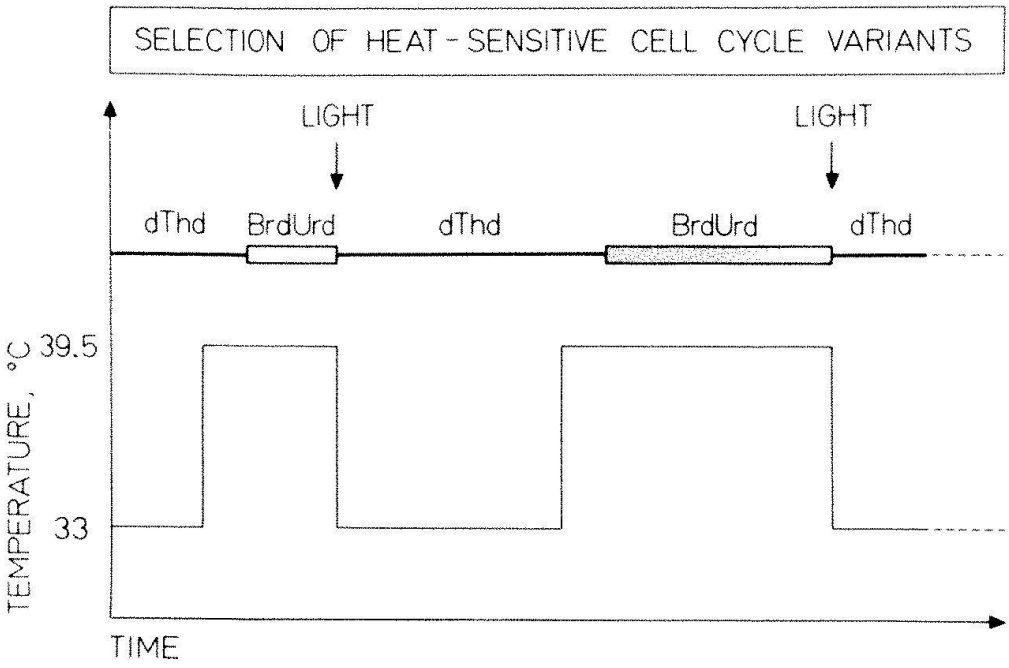

Fig. 3. General procedure used for selection of heat-sensitive cell-cycle mutants based on incubation with 5-bromodeoxyuridine (BrdUrd) at the nonpermissive temperature, followed by irradiation with light (wave length: 400 nm) and incubation of surviving cells at the permissive temperature with thymidine (dThd).

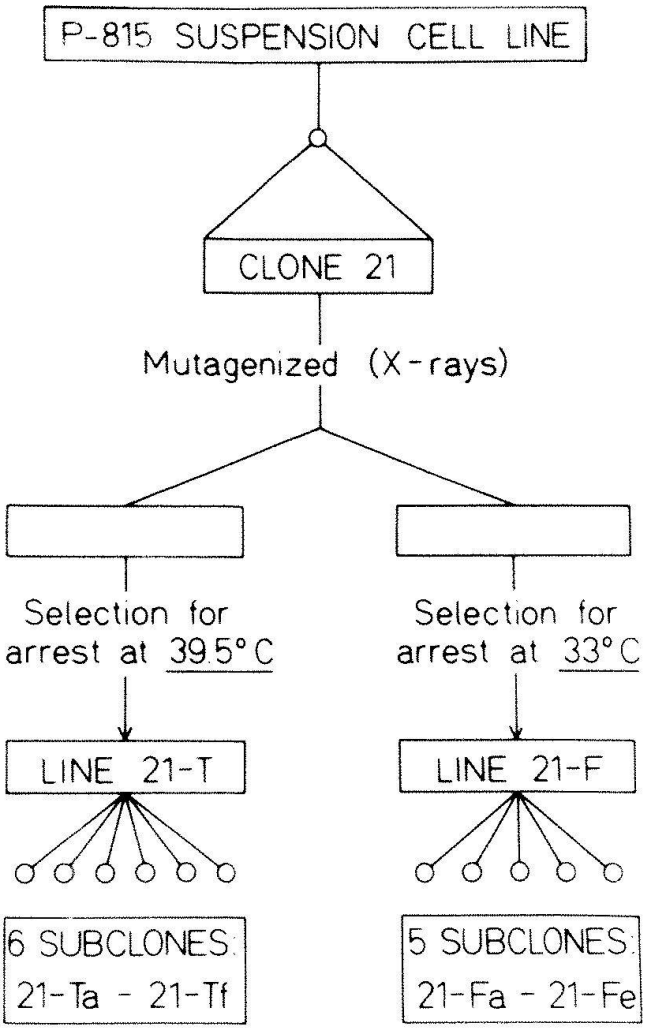

Fig. 4. Selection and nomenclature of heat- and cold-sensitive cell-cycle mutants of the P-815 mastocytoma line.

tive (hs, multiplying at 33° C, arrested at 39.5° C) and cold-sensitive (cs, multiplying at 39.5° C, arrested at 33° C) cell-cycle mutants as follows (fig. 4). A clonal subline (termed clone 21 or K 21 and representing the "wild-type" control) of the P-815 mastocytoma was subjected to mutagenization by treating 2.5×10^7 cells with 250 rads of X-rays. The culture was then split and subjected to the selection procedure for hs and cs mutants described above. Of the two variant cell lines (21-T and 21-F) thus obtained, clonal sublines were derived by isolation of single cells. The six hs subclones to be used in further studies were designated as 21-Ta – 21-Tf, while the five cs subclones were designated as 21-Fa – 21-Fe.

The results on cell multiplication of the hs subclones 21-Ta – 21-Tf and of the wild-type K 21 line at 33° C and 39.5° C are illustrated in figure 5. Whereas K 21 cells exhibited exponential proliferation at both incubation temperatures, multiplication of all six 21-T subclones nearly ceased after the first 24 hours at 39.5° C. On the other hand, multiplication of 21-T cells at 33° C was similar to that of K 21 cells. After 6–12 days at 39.5° C, cell multiplication of 21-T cells was resumed. This was shown to reflect the outgrowth of revertants with properties similar to those of K 21 cells. The five cs 21-F subclones exhibited exponential cell multiplication at 39.5°

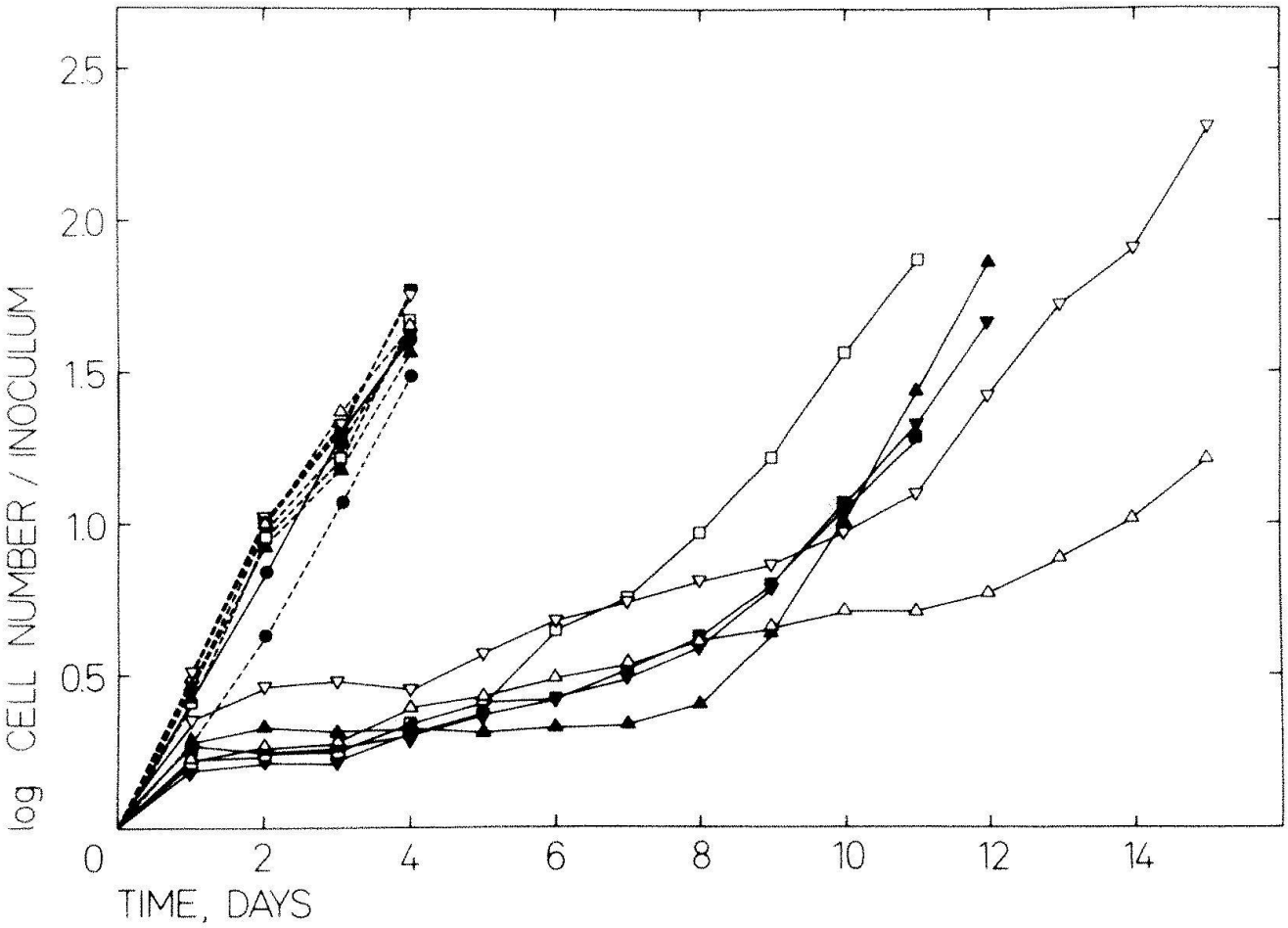

Fig. 5. Multiplication of "wild-type" K 21 cells and of 6 subclones of the hs 21-T line at 33 °C (———) and at 39.5 °C (– – –).

● K 21
△ 21-Ta
▲ 21-Tb
▽ 21-Tc
▼ 21-Td
□ 21-Te
■ 21-Tf

C, whereas the cell multiplication rate decreased to minimal levels after the first 3 days of incubation at 33° C (data not shown).

For determination of cell-cycle distribution, cells were prepared for cytofluorometric determination of DNA content as described by Grieder et al. (1975). Typical results obtained for the hs subclone 21-Tb are presented in figure 6. It is seen that at the time when the cells were transferred from the permissive temperature of 33° C to 39.5° C, their distribution in the cell cycle was typical of that of an exponentially multiplying population, with two peaks corresponding to cells in G_1 phase and in G_2 phase and mitosis, respectively, which are connected by a broad distribution of cells in S phase with intermediate DNA values. After 8 hours of incubation at 39.5° C, the percentage of cells in G_1 phase already had increased, and after 24 hours at the nonpermissive temperature, nearly all cells had a DNA content typical of G_1 phase. It may, therefore, be concluded that at 39.5° C these cells were arrested between mitosis and S phase and thus may be considered to be true cell-cycle mutants. Similar results were obtained by cytofluorometric analysis of the other 21-T subclones and after transfer of the cs 21-F subclones to the nonpermissive temperature of 33° C. The arrest of cell multiplication of 21-T and 21-F cells at the respective nonpermissive temperature was reversible, i.e. cell multiplication was resumed within 24 hours if cells were brought back to the permissive temperature. Thus, these results indicate that at the nonpermissive temperature, both the hs

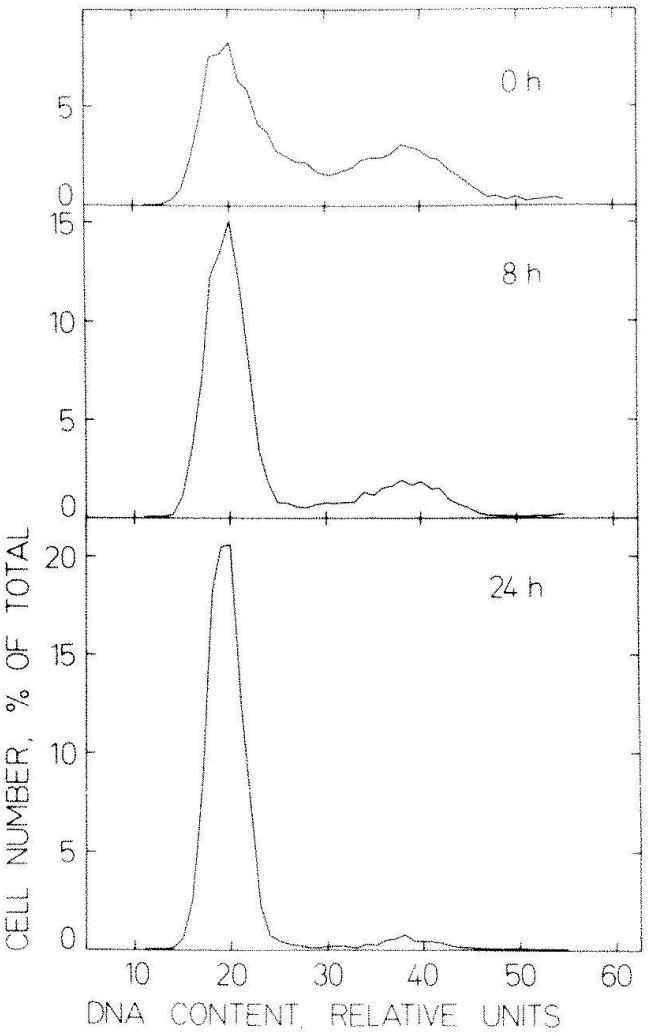

Fig. 6. Cell cycle position of 21-Tb cells, as determined by cytofluorometric measurements of cellular DNA content, at different times after transfer of cells from 33°C to 39.5°C.

and the cs cells accumulated in a state of reversible proliferative quiescence which is located between mitosis and S phase.

To determine if the hs and cs phenotypes of the 21-T and 21-F cell-cycle mutants are expressed in a dominant or recessive manner, the mutant cells were arrested at the nonpermissive temperature and fused to "wild-type" (WT) P-815 cells that had been arrested by incubation in culture medium containing a low concentration (0.01%) of serum. As a means of identifying homokaryons and heterokaryons, the cells were induced to take up latex particles of different sizes. For cell-to-cell fusion, a method based on the effects of polyethylene glycol and dimethyl sulfoxide, as described in detail elsewhere (Zimmermann et al., 1981), was used. After fusion, the cells were reincubated at the respective nonpermissive temperature in medium containing 10% serum and [^{3}H]thymidine. At different times, DNA-synthetic activity of mono- and binuclear cells was determined by autoradiography. As illustrated schematically in figure 7, the 21-T x WT heterokaryons entered the S phase under these conditions, indicating that the hs 21-T phenotype was recessive, whereas the cs 21-F cells behaved in a dominant manner, i.e. the heterokaryons did not enter the S phase for at least 48 hours, as reported in detail elsewhere (Zimmermann et al., 1981). These results are compatible with the assumption that the hs 21-T cells contain a heat-labile gene product which is essential for traverse through G_1 phase, and which is inactivated upon shift to 39.5° C, resulting in proliferative quiescence of cells with a DNA content typical of G_1 phase. On the other hand, the dominant expression of the cs phenotype of 21-F cells supports the assumption that these cells contain a heat-labile gene product which in its "active" form (i.e. the form present at 33° C) induces a state of proliferative quiescence which is also located between mitosis and S phase.

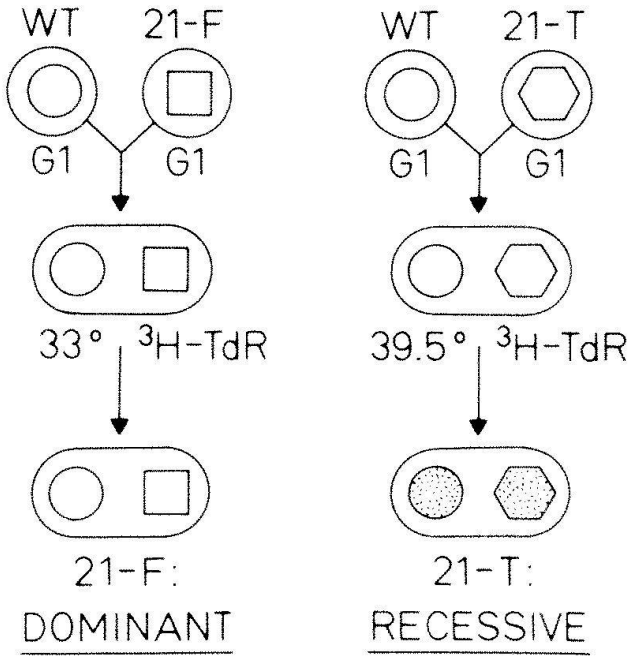

Fig. 7. Autoradiographic analysis of the capacity to enter S phase after fusion of "wild-type" (WT) cells arrested by serum deprivation with cs 21-F cells arrested at 33°C or hs 21-T cells arrested at 39.5°C. After cell-to-cell fusion, the cultures were incubated at the temperature indicated in medium containing [^{3}H]-thymidine (^{3}H-TdR) and 10% serum. DNA-synthesizing nuclei (i.e. covered by silver grains formed by decay of ^{3}H during autoradiography) are depicted by the black dots.

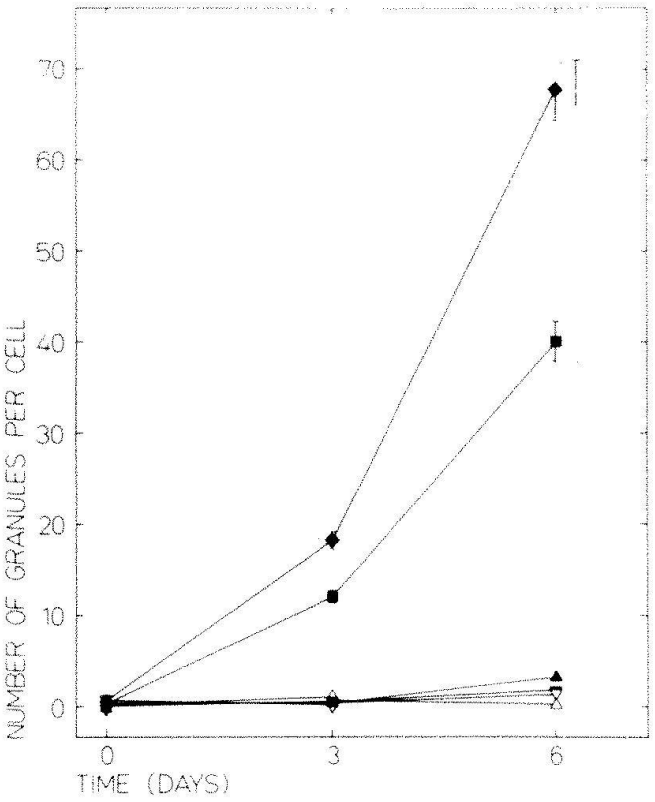

Fig. 8. Formation of metachromatically staining mast-cell granules by cs and hs cell-cycle mutants and "wild-type" (K 21) mastocytoma cells at two different temperatures.
△ K 21 cells at 33 °C
▽ K 21 cells at 39.5 °C
◆ 21-Fb cells at 33 °C
■ 21-Fc cells at 33 °C
▲ 21-Tb cells at 39.5 °C
▼ 21-Tf cells at 39.5 °C

As an additional possibility to characterize the states of proliferative quiescence attained by 21-T and 21-F cells at the respective nonpermissive temperature, the degree of cellular differentiation of these mutants of the mastocytoma line was evaluated after staining of fixed cells with toluidine blue. With this procedure, heparin-containing granules which are a typical constituent of normal mast cells may be identified by their metachromatic staining properties. As seen in figure 8, "wild-type" K 21 cells contained very few, if any, metachromatically staining granules, and similar results were obtained for 21-Tb and 21-Tf cells during culture at 33° C and after shift to 39.5° C. On the other hand, upon incubation of 21-Fb and 21-Fc cells at the nonpermissive temperature of 33° C, the average number of mast-cell granules per cell increased markedly.

In conclusion, the results obtained, as summarized in table 1, are compatible with the assumption that the gene product responsible for the cs phenotype of 21-F cells may be a pleiotropic effector inducing both a state of reversible proliferative quiescence and the formation of mast-cell granules, i.e. specialized cellular organelles, during incubation at 33° C, while at 39.5° C, this gene product would be inactive. On the other hand, lack of activity of a gene product required for traverse through G_1 phase, as exemplified by the hs 21-T cell-cycle mutants at 39.5° C, apparently is insufficient for induction of mast-cell differentiation. It may, therefore, be appropriate to distinguish between qualitatively different states of proliferative quiescence. The state of quiescence induced in 21-F cells at 33° C, being characterized by (a) dominant expression in cell fusion experiments and (b) cellular differentiation, may represent a useful model system because mechanisms similar to those responsible for induction of proliferative quiescence and cellular differentiation in 21-F cells at 33° C may be operative also in normal cells. The mutation underlying the cs phenotype of 21-F cells may thus be a means to identify one of the elements in the regulation of the transition of cells from exponential proliferation into a state of proliferative quiescence.

Table 1. Characteristics of heat- and cold-sensitive cell-cycle mutants of the P-815 murine mastocytoma line.

	hs mutants (21-T)	cs mutants (21-F)
Cell cycle arrest at	39.5 °C	33 °C
Cell proliferation at	33 °C	39.5 °C
Arrested in	G_1	G_1 (or "G_0")
Reversibility of arrest	reversible	reversible
Expression of ts phenotype in heterokaryons	recessive	dominant
Cell differentiation of arrested cells	no	yes
Cell cycle arrest induced by	absence of active gene product	presence of active gene product (pleiotropic effector?)

References

Baserga, R. 1978: Resting cells and the G_1 phase of the cell cycle. Journal of Cellular Physiology *95*, 377–386.

Basilico, C. 1978: Selective production of cell cycle specific ts mutants. Journal of Cellular Physiology *95*, 367–376.

Dunn, T.B., Potter, M. 1957: A transplantable mast-cell neoplasm in the mouse. Journal of the National Cancer Institute *18*, 587–601.

Green, J.P., Day, M. 1960: Heparin, 5-hydroxytryptamine, and histamine in neoplastic mast cells. Biochemical Pharmacology *3*, 190–205.

Grieder, A., Schleisner, P., Maurer, R. 1975: Chemical and pulse-cytophotometric measurements of DNA and RNA in cultured cells treated with cytostatic drugs. In: Schumann, J., Göhde, W., Büchner, T., eds.: Pulse-cytophotometry, part II, pp. 161–171. European Press, Ghent.

Prescott, D.M. 1976: Reproduction of eukaryotic cells. Academic Press, New York.

Puck, T.T., Kao, F. 1967: Genetics of somatic mammalian cells. V. Treatment with 5-bromodeoxyuridine and visible light for isolation of nutritionally deficient mutants. Proceedings of the National Academy of Sciences USA *58*, 1227–1234.

Schindler, R., Day, M., Fischer, G.A. 1959: Culture of neoplastic mast cells and their synthesis of 5-hydroxytryptamine and histamine in vitro. Cancer Research *19*, 47–51.

Simchen, G. 1978: Cell cycle mutants. Annual Review of Genetics *12*, 161–191.

Siminovitch, L., Thompson, L.H. 1978: The nature of conditionally lethal temperature-sensitive mutations in somatic cells. Journal of Cellular Physiology *95*, 361–366.

Tobey, R.A., Crissman, H.A. 1975: Unique techniques for cell cycle analysis utilizing mithramycin and flow microfluorometry. Experimental Cell Research *93*, 235–239.

Zimmermann, A., Schaer, J.C., Schneider, J., Mölo, P., Schindler, R. 1981: Dominant versus recessive behavior of a cold- and a heat-sensitive mammalian cell cycle variant in heterokaryons. Somatic Cell Genetics *7*, 591–601.

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The differentiated phenotype of the transformed cell in leukemias

Jean-François Conscience

Abstract

Data are reviewed from several types of leukemias to show that:
1) Leukemic cells share with normal hemopoietic precursor cells differentiation markers with a high degree of lineage fidelity.
2) When leukemic cells can be induced to differentiate, they mature into essentially normal blood cells of the corresponding lineage.
3) When a direct biochemical and molecular analysis of leukemic cells and of pure populations of hemopoietic precursor cells is possible, few, if any, differences are found in the expression of differentiated functions.
These results support a model of leukemogenesis whereby cell transformation affects primarily the mechanisms controling proliferation of normal hemopoietic precursor cells, without altering significantly the expression of differentiated functions. A consequence of the model is that a relatively limited number of changes in the pattern of gene expression, most likely affecting only the hormonal control of growth, is enough to account for most, if not all, of the malignant properties of leukemic cells. The model has important implications for leukemia therapy and for understanding the functional significance of the recently defined oncogenes.

Zusammenfassung

Jüngste Erkenntnisse, die man über verschiedene Typen leukämischer Zellen gewonnen hat, zeigen:
1) Die Differenzierungsmerkmale der leukämischen Zellen stimmen mit jenen der entsprechenden hämopoietischen Vorläuferzellen weitgehend überein.
2) Wenn leukämische Zellen veranlasst werden, sich zu differenzieren, entstehen im wesentlichen normale Blutzellen.
3) Wenn eine biochemische und molekulare Analyse von leukämischen Zellen und von reinen Populationen hämopoietischer Vorläuferzellen möglich ist, werden, wenn überhaupt, nur geringe Unterschiede in den differenzierten Funktionen gefunden.
Diese Ergebnisse stützen ein Modell von Leukämogenese, wobei Zelltransformation in erster Linie jene Mechanismen beeinflusst, welche die Proliferation von normalen hämopoietischen Vorläuferzellen kontrollieren, ohne auf den Ausdruck der differenzierten Funktionen einzuwirken. Eine Folge des Modells ist, dass eine relativ begrenzte Zahl von Änderungen in der Genaktivität, wahrscheinlich nur die hormonale Kontrolle des Wachstums berührend, für die meisten, wenn nicht für alle, bösartigen Eigenschaften der leukämischen Zellen verantwortlich ist. Das Modell hat wichtige Auswirkungen auf die Therapie der Leukämie und hilft, die funktionale Bedeutung der kürzlich definierten Oncogene besser zu verstehen.

Transformed cells, such as those isolated from leukemias, often express differentiated functions (Wigley, 1975). For example, many plasmocytomas produce immunoglobulins, Friend erythroleukemic cells can be induced to make hemoglobin, and many lines of basophilic leukemia cells contain histamine. The expression of tissue-specific traits has been an invaluable help in classifying the many forms of leukemias and has also led to the use of leukemia cell lines as *in vitro* models for normal hemopoietic cell differentiation.
Nevertheless, leukemic cells obviously differ in many respects from normal blood cells. Not all functions of the mature cells are found in transformed cells, and those that are expressed usually exhibit levels different

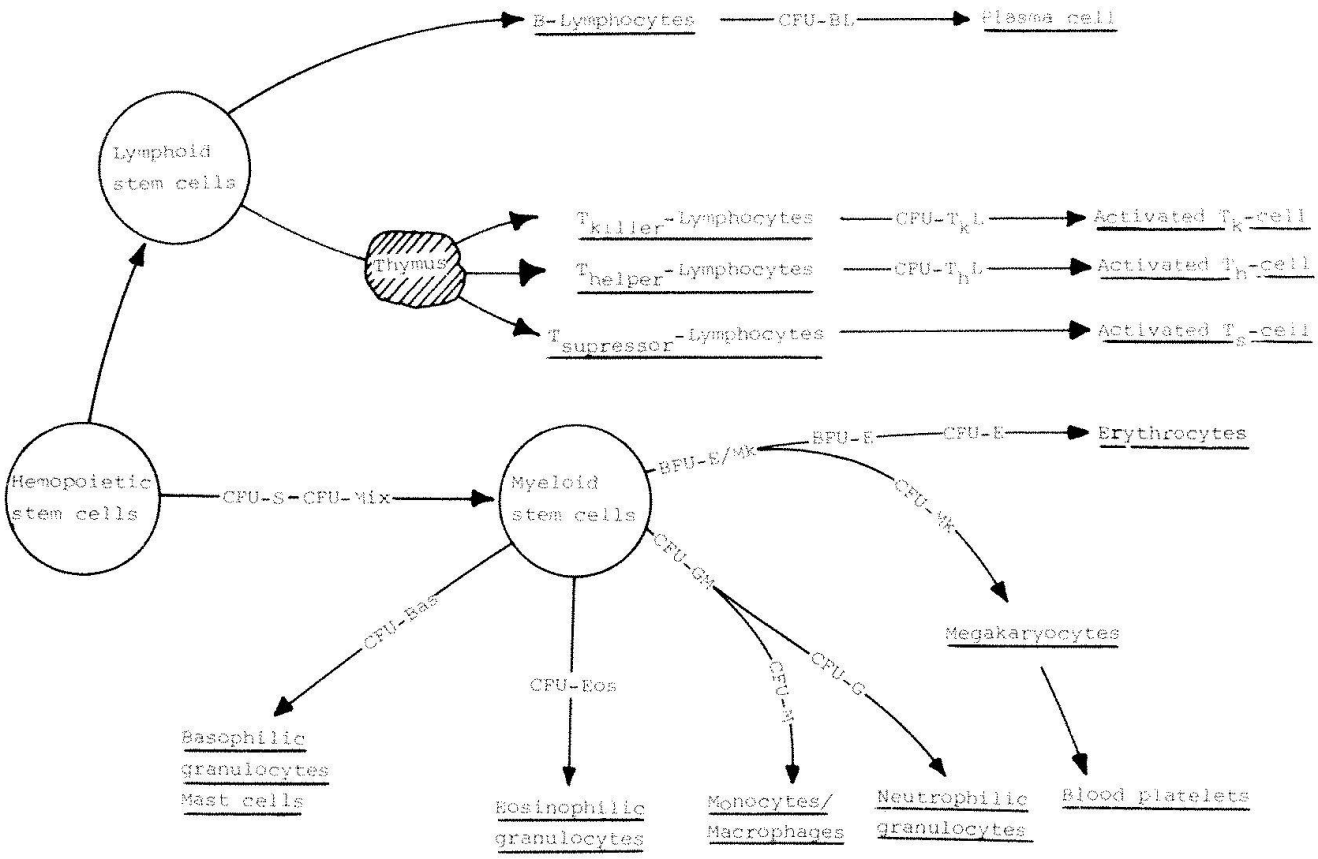

Fig. 1. Normal hemopoiesis. Underlined names designate mature, end-stage cells which are morphologically identifiable. Colony-forming units (CFU's) and burst-forming units (BFU's) represent, in the various lineages, proliferating precursor cells which can be assayed functionally (E, erythroid; Mk, megakaryocytic; M, monocytic; G, neutrophilic; Eos, eosinophilic; Bas, basophilic; BL, B-lymphocytic; TL, T-lymphocytic; CFU-S, pluripotent stem cell as assayed in vivo; CFU-Mix, pluripotent stem cell as assayed in vitro).

from those of mature blood cells. Furthermore, leukemic cells can express functions which are not found in their normal counterparts. These "extra" functions often involve the coexpression of markers typical of more than one hemopoietic lineage or the expression of molecules found in embryonic cells. Finally, leukemic cells proliferate *in vivo* and *in vitro,* a property that mature blood cells have either lost altogether or retained under very tight hormonal control. At a time when little was known about normal hemopoiesis, these observations led to the conclusion that leukemic cell transformation was a highly pleiotropic process affecting a whole range of functions related both to cell growth and to cell differentiation, and resulting in a very abnormal, dedifferentiated cell that bears little relationship to normal cells (Wigley, 1975; Ibsen and Fishman, 1979).

In the last two decades, however, considerable progress has been made toward understanding normal hemopoietic cell differentiation (Till and McCulloch, 1980) and this has led to a rethinking about the nature of the leukemic cell. Thanks to the design of novel morphological, cytochemical and immunocytochemical techniques, better identification of blood cells and of their immediate progenitors is now possible. More importantly, the development of colony assays *in vivo* and *in vitro* now provides an indirect functional analysis of several types of precursor cells (Metcalf, 1977). As a result, we know now that all the cellular elements of the blood arise from a limited number of pluripotent stem cells which persist throughout the life of an individual and from which cohorts of proliferating and differentiating cells arise continuously in response to physiological needs. The salient features of this process are outlined in figure 1. Four aspects of the system should be kept in mind. First, stem cells are relatively undifferentiated and specific functions appear progressively dur-

ing differentiation. Second, successive commitment steps restrict progressively the differentiation capabilities of the stem cells, so that, for example, transient, bipotential cells appear (CFU-GM in fig. 1). Third, cell proliferation accompanies differentiation all the way to the mature end-stage cell, in which this property either disappears (e.g. erythrocytes, granulocytes) or is subject to very specific triggers (e.g. macrophages, lymphocytes). Fourth, certain stages of hemopoiesis occur outside the bone marrow in specialized organs such as the thymus, or in the various tissues of the body (e.g. mast cell maturation), implying that certain normal hemopoietic precursor cells have the capacity to migrate and divide outside of their tissues of origin.

Table 1. Distribution of MBM-1, a mouse differentiation marker defined by a rat monoclonal antibody, on the surface of hemopoietic cells.

Cell type	Presence of MBM-1
Pluripotent stem cells	
assayed in vitro (CFU-Mix)	all negative
assayed in vivo (CFU-S)	all negative
Biopotential neutrophil-monocyte precursor cells (CFU-GM)	40 % positive cells
Neutrophil precursor cells (CFU-G)	40 % positive cells
Monocyte precursor cells (CFU-M)	65 % positive cells
Peritoneal macrophages	8 % positive cells
Blood neutrophils	all positive
Eosinophil granulocytes	all positive
Peritoneal mast cells	all negative
Early erythroid precursor cells (BFU-E)	all negative
Late erythroid precursor cells (CFU-E)	all negative
Erythroblasts, erythrocytes	all negative
B-lymphocytes (spleen and lymph nodes)	all negative
T-lymphocytes (spleen)	55 % positive cells

The data are based on immunofluorescent staining of various hemopoietic organs and analysis of cell fractions obtained after fluorescence-activated, antibody-mediated cell sorting of bone marrow cells, using hematological staining procedures for mature cells and functional assays for progenitors. For details, see Davis et al. (1983).
MBM-1 is present on cells of the myeloid series (neutrophil-monocyte pathway of differentiation) and on a subpopulation of T-lymphocytes. The nature of this antigen is unknown.

With these considerations in mind, it is evident that many properties of leukemic cells, including some that appear to be highly abnormal, are, in fact, properties of normal hemopoietic precursor cells, such as the various CFU's, BFU's and stem cells listed in figure 1. Hence the new model of leukemogenesis postulates that cell transformation uncouples the mechanisms for control of proliferation and differentiation in hemopoietic progenitors (Greaves, 1982; Till, 1982; Warner, 1982). As a result, leukemic cells are generated with a capacity for unlimited self-renewal and a greatly decreased capacity for terminal differentiation. This does not require a major reorganization of the pattern of gene expression in the leukemic cell, which remains largely the same as in the corresponding non-transformed precursor cell. This model leads to the following predictions: (1) Leukemic cells should display differentiation markers of normal hemopoietic precursor cells with a high degree of lineage fidelity; (2) If leukemic cells can be induced to differentiate terminally, they should generate essentially normal end-stage cells; (3) A direct comparison between leukemic cells and their normal counterparts should fail to reveal any significant differences in the expression of differentiated functions. In this report, I shall present recent results from several laboratories, including my own, which are in agreement with these predictions.

Antibodies reacting with specific markers of hemopoietic cell differentiation are becoming available in increasing numbers, thanks to the development of the hybridoma technology (Kennett et al., 1980). In this approach, rats or mice are innoculated with cells from allogeneic hemopoietic organs. Antibodies are thus generated against the many antigenic molecules present on the different cell types and the different maturation stages found in the innoculum. Following fusion of the immunized spleen cells with a myeloma cell line, the resulting hybridomas can be selected and cloned, and the cell specificity of the antibodies that some of them produce can be established with standard im-

munological techniques and antibody-mediated cell sorting. Using such a strategy, we have isolated rat monoclonal antibodies reacting with various subpopulations of mouse bone marrow cells, and one of these reagents has been characterized more fully (Davis et al., 1983). The surface antigen it recognizes, MBM-1, is found on a number of normal hemopoietic cells, as summarized in table 1. When a series of cell lines, established from different types of leukemias, are screened with the same antibody, the results given in table 2 are obtained. In all cases, the expression of MBM-1 in leukemic cells is consistent with lineage fidelity and no evidence for an aberrant expression can be found. The results of more extensive studies of the same type (Greaves, 1982; Warner, 1982), using human and murine leukemic cells or cell lines and a large panel of monoclonal antibodies directed against lymphocyte differentiation markers, are entirely consistent with the model of a precursor cell origin of leukemias and fail to support the idea of dedifferentiation or gross aberrant gene expression.

Leukemic cells, under certain circumstances, are capable of generating normal mature cells. This has now been shown in several types of leukemias. Friend erythroleukemia cells in culture express a set of differentiated functions that place them around the CFU-E stage (see fig. 1) of erythroid maturation (Marks and Rifkind, 1978). When treated with various chemicals, such as dimethylsulfoxide or sodium butyrate, these cells undergo a number of changes. Using several enzymatic markers of erythroid differentiation, whose changes *in vivo* have been documented (Denton et al., 1975; Setchenska and Arnstein, 1978), it is possible to show that Friend cell differentiation follows essentially a normal pathway, as summarized in figure 2 (Conscience and Meier, 1980; Conscience and Meier, 1980). Although established Friend cell lines do not enucleate well *in vitro,* one often finds among newly isolated Friend cell lines (Conscience et al., 1982) clones containing a relatively large proportion of enucleating cells, following chemical induction of differentiation. An analysis of these enucleating cells (Deslex, 1982), using single cell immunostaining procedures, shows that differentiated Friend cells undergo membrane rearrangements similar to those occuring *in vivo* during enucleation of the orthochromatic erythroblast (Geiduschek and Singer, 1979). These findings are shown in figure 3. Moreover, in improved culture conditions, established Friend cell clones also appear to enucleate normally (Volloch and Housman, 1982). Certain murine myeloid and lymphoid leukemia cell lines are similarly capable of undergoing normal terminal differentiation, when treated with various effectors (Sachs, 1978; Sachs, 1980; Ralph et al., 1982). Finally, the most convincing evidence available today showing that leukemic cells can mature into normal end-stage cells comes from work done with a temperature-sensitive mutant of avian erythroblastosis virus (AEV) (Beug et al., 1982). When grown at a temperature at which the virally coded transforming protein is functional, AEV-transformed cells display properties of immature chicken erythroblasts. If the temperature is raised to 42° C, the transforming protein becomes inactive and the cells differentiate irreversibly within a few cell divisions over a few days into perfectly normal chicken erythrocytes.

A direct biochemical and molecular comparison between transformed leukemic cells and the corresponding normal precursor

Table 2. Distribution of MBM-1, a mouse hemopoietic differentiation marker defined by a rat monoclonal antibody, in several leukemia cell lines.

Leukemia cell line	Lineage and /or cell phenotype	Presence of MBM-1
Friend cells (F46N)	erythroid	—
426C	myelomonocytic	+
427E	myelomonocytic	+
WEHI-3	myelomonocytic	+
P3.X63.Ag8.6.5.3	myeloma	—
WEHI-231	B-lymphoma	—
WEHI-279	B-lymphoma	—
707/3	pre B-cell	—
18.81	pre B-cell	—
136.5	thymoma	+
PB-1	mast cell	—

Comparison with the data shown in Table 1 reveals that expression of MBM-1 is restricted to leukemic cells related to lineages where the antigen is normally expressed. For the origin of the lines listed here and the indirect immunofluorescent technique used, see Davis et al. (1983).

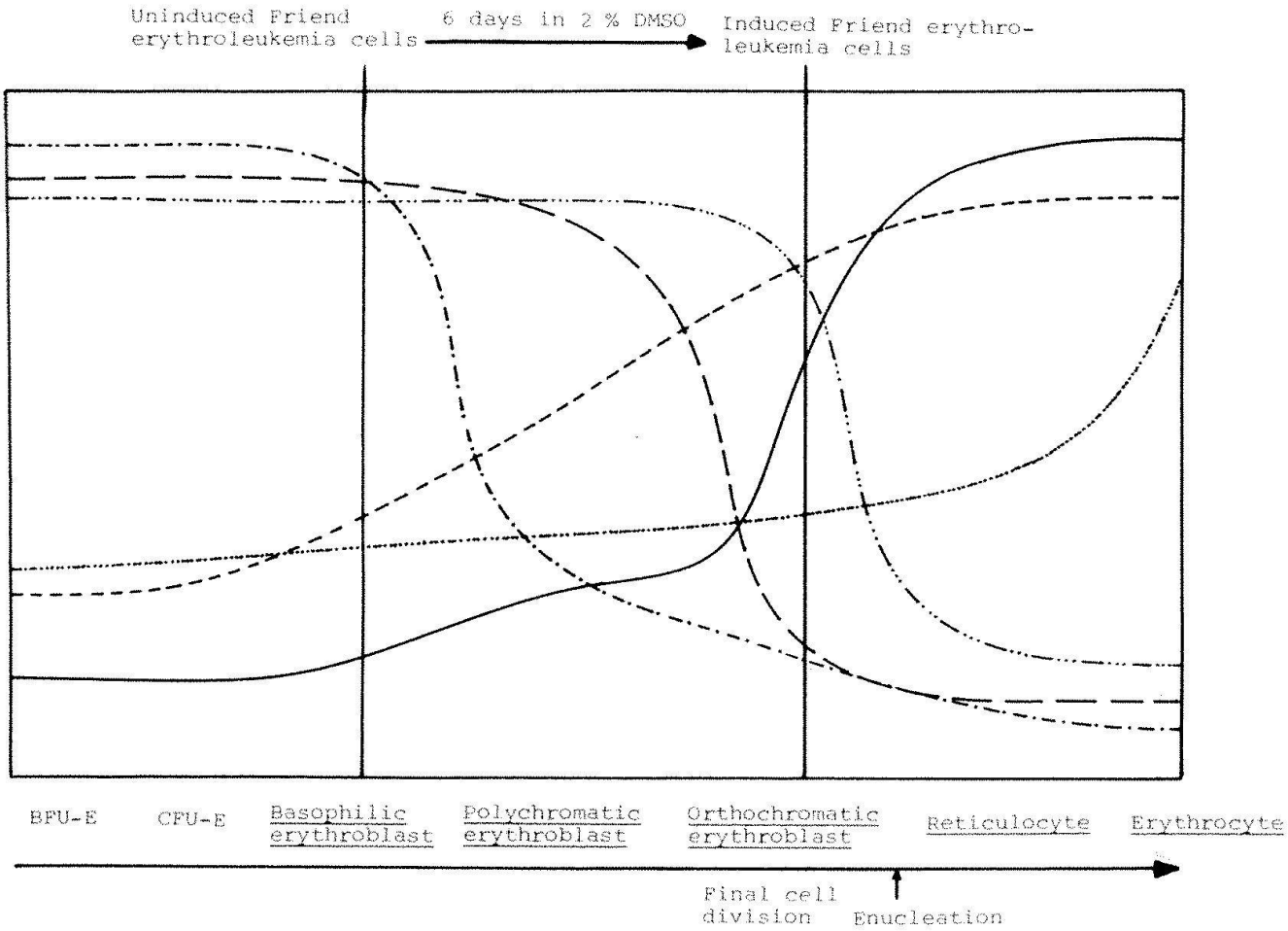

Fig. 2. Comparison between normal erythroid differentiation in vivo and chemically-induced Friend erythroleukemia cell differentiation in vitro. The plots (——— hemoglobin; · · · catalase; ——— 6-phosphogluconate dehydrogenase; --- carbonic anhydrase; ·-·-· lactate dehydrogenase; ·-·-·-·-· glucose-6-phosphate dehydrogenase) illustrate the changes in specific activities (ordinate, arbitrary scale) that take place in vivo during late erythroid differentiation. They have been compiled from the data of Denton et al. (1975) and Setchenska and Arnstein (1978). At the bottom of the figure are listed a few intermediate cell types of erythropoiesis. As in Fig. 1, underlined names designate morphologically identifiable cells, whereas CFU-E (colony-forming unit-erythroid) and BFU-E (burst-forming unit-erythroid) represent late and early erythroid progenitors, respectively, that can be assayed functionally.
When the same markers are followed in Friend erythroleukemia cells during chemically-induced differentiation, as indicated at the top of the figure, the same pattern of changes is observed: hemoglobin and carbonic anhydrase increase, lactate dehydrogenase and 6-phosphogluconate dehydrogenase decrease, and glucose-6-phosphate dehydrogenase and catalase do not change (for details, see Conscience and Meier [1980] and Conscience and Meier [1980]). This is taken as evidence for the view that Friend cells, upon induction, resume an essentially normal program of erythroid differentiation.

cells is only possible when the latter can be cultured as pure populations and when malignant cells arise in such cultures by cell transformation. Such a situation has recently been observed in our laboratory (Ball et al., 1983). Like others (Galli et al., 1982), we have been able to grow lines of basophil-/mast cell precursors, by exposing mouse bone marrow cells to the appropriate growth factors (contained in media conditioned either by pokeweed mitogen-stimulated spleen cells or by the WEHI-3 myelomonocytic leukemia cell line). Unexpectedly, nine months after its isolation, one of these lines transformed spontaneously. As shown in table 3, non-transformed basophil/mast cell lines require the continuous presence of growth factors for proliferation *in vitro* and for cloning. The transformed cells do not. In addition, the normal lines fail to give rise to tumors *in vivo,* while the transformed cells are highly tumorigenic and invasive in syngeneic animals. In spite of these dramatic differences in growth properties, the trans-

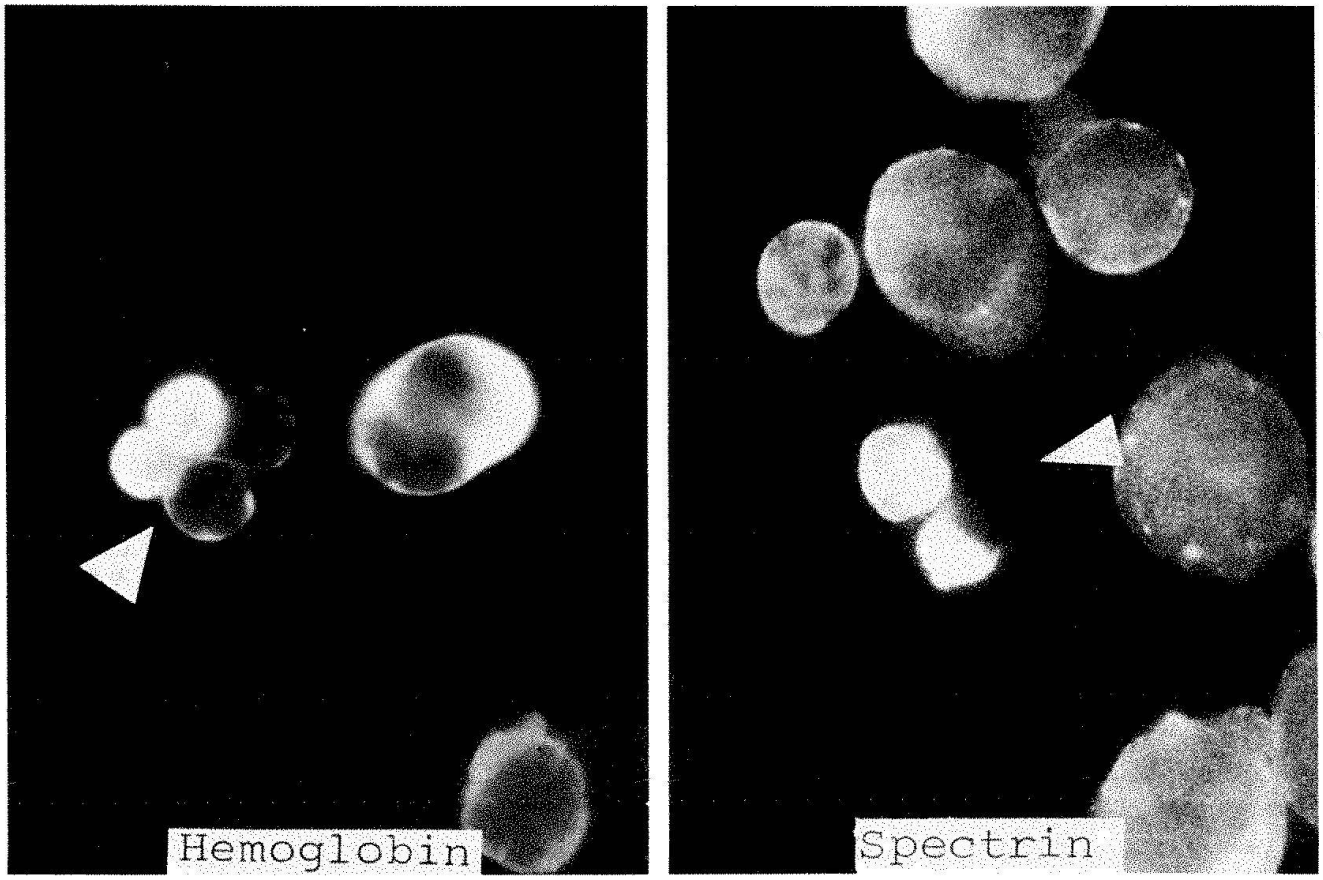

Fig. 3. Enucleating erythroblast-like cells in a newly-transformed Friend erythroleukemia cell line, after 4 days in 1.3 % dimethylsulfoxide. Staining was done by an indirect immunofluorescence technique, using rabbit anti-mouse hemoglobin and anti-mouse spectrin antisera, as described in Conscience et al. (1982).
After staining for hemoglobin, two enucleating cells (arrowhead) display a bright cytoplasmic half (the future reticulocyte) and a dark nuclear vesicle, surrounded by a thin ring of fluorescence. The latter stems from the small amount of hemoglobin-containing cytoplasm, trapped between the nucleus and the section of the plasma membrane that surrounds it.
After staining for spectrin, the nuclear side of the enucleating cells (arrowhead) remains totally unstained, indicating that all the spectrin molecules in the plasma membrane have been translocated into the cytoplasmic side of the cells.
A similar rearrangement is known to occur in normal enucleating erythroblasts (Geiduschek and Singer, 1979) and is taken here as additional evidence for the apparent normalcy of Friend cell differentiation in vitro.

Table 3. Growth properties of transformed and non-transformed basophil/mast cell precursors grown in vitro with and without the addition of growth factors.

	Non-transformed cells	Transformed cells
Population doubling time without factors	die	28 hours
Population doubling time with factors	40 hours	23 hours
Cloning efficiency without factors	0 %	6 %
Cloning efficiency with factors	4 %	80 %
Tumorigenicity and invasiveness in vivo	no	yes

For details, see Ball et al. (1983).

formed cells cannot be distinguished from their normal counterparts with respect to the expression of differentiated functions: both cell types express the same markers, to a similar extent, and look like immature mast cells (table 4). Thus, in this instance, transformation clearly does not shift the cell population toward a different state of differentiation. There is also no evidence for a complete maturational block in the transformed cells. These, like their untransformed counterparts, display a striking cellular heterogeneity in the degree with which basophil/mast cell traits are expressed. The same heterogeneity persists after cloning of both types of cell lines. For example, the number of Toluidine Blue-positive granules per cell varies considerably, as well as the de-

gree of metachromasia of each granule. This suggests that cells at different stages of maturation are present and that a reduced capacity to undergo normal terminal differentiation persists in these cultures. This has been recently established in the case of the non-transformed cells (Galli et al., 1982). Interestingly enough, the transformed cells remain diploid, so that tumor formation in this system is not associated with the emergence of gross chromosomal abnormalities, and, moreover, they still respond to the addition of growth factors by an increase in growth rate and cloning efficiency (tab. 3).

In summary, the only significant cellular change that can be correlated with malignancy in the transformed basophil/mast cell cultures is their loss of requirement for added growth factors in vitro. This observation supports a classical hypothesis (Holley, 1972) relating the abnormal growth properties of malignant cells to a decreased sensitivity toward natural regulators. It is obviously unclear, at this stage, whether the growth factor independence *in vitro* reflects a similarly altered response towards endogeneous growth promoting and inhibiting substances.

Table 4. Differentiated traits expressed in culture by transformed and non-transformed basophil/mast cell precursors grown in vitro.

Differentiated traits	Non-transformed cells	Transformed cells
Basophilic granules with May-Grünwald-Giemsa	+	+
Metachromatic granules with Toluidine Blue	+	+
Astra Blue staining	+	+
Staining for chloro-acetate-esterase	+	+
Staining for aminopeptidase	+	+
Histamine content (pg/cell)	0.022 ±0.002	0.15 ±0.02
Number of IgE receptor sites per cell	1.2×10^5	2.3×10^5

The staining reactions of both cell types are less intense than those of mature mast cells and display heterogeneity from cell to cell. Transformed cells consistently exhibit higher amounts of histamine, but both values are much lower than those of mature mast cells. For details, see Ball et al. (1983).

If so, however, it might be a sufficient alteration to explain the tumoral growth of these cells *in vivo,* since normal basophil/mast cell precursors are known to leave the bone marrow and "invade" the tissues, where they undergo terminal differentiation and limited proliferation (Matsuda et al., 1981).

The data reviewed here support the hypothesis that leukemic cells are functionally equivalent to normal hemopoietic precursor cells with an altered regulation of cell division. It therefore appears that few cellular changes are required to effect transformation of the normal precursor cells. While the nature of these changes is still obscure, the results obtained by comparing directly normal and transformed basophil/mast cell precursors suggest clearly that cell transformation affects primarily the hormonal controls of cell proliferation and leaves intact the expression of differentiated functions.

Such a model of leukemogenesis presents some important implications. The similarity of leukemic cells to normal hemopoietic progenitors provides a rationale for their use as models for studying certain aspects of normal development. In particular, they can be used as immunogens with a reasonable hope of obtaining lineage-specific and/or differentiation stage-specific monoclonal antibodies. These, in turn, can be used to better characterize and purify the corresponding normal precursor cell, which, in most cases, still remains an elusive entity. Such an approach could be used therapeutically to enrich for certain cell types and/or delete others in the bone marrow cell populations prepared for transplantation into radiation-treated leukemic patients, thus decreasing the immunological hazards currently associated with the procedure. Since leukemic cells *in vitro* can often be induced to differentiate terminally into normal, non-malignant mature cells, another therapeutic application could consists in trying to achieve the same results *in vivo.* Preliminary attempts along these lines have been disappointing so far, but the use of specific monoclonal antibodies might allow for a better targeting of agents known to promote such differentiation (Moore and Sheridan, 1982).

A better characterization of the functional changes leading to a leukemic cell goes to-

gether with a better understanding of the genetics of the process. As reviewed elsewhere in this volume, oncogenes that are either abnormal or abnormally expressed have been found associated with a number of tumors and leukemias. Several of them have been cloned and sequenced. Thus, the genetics of these entities is now far advanced, but their physiological significance to the normal and the transformed cells is still obscure. I hope that model systems like the transformed and non-transformed basophil-/mast cell lines described here, where a direct biochemical and molecular comparison of the two cell types is possible, will prove useful in narrowing down the possibilities and in providing for adequate experimental verification of hypotheses.

Updating note

Using the basophil/mast cell lines described above, we have obtained now results that support many of the ideas expressed in the preceeding review:

1. The factor required for the proliferation of the non-malignant cells in interleukin-3 (IL-3; Conscience and Iscove, unpublished results).
2. Factor-independent proliferation in vitro and tumorigenicity in vivo remain associated in somatic cell hybrids between malignant and non-malignant cells, in agreement with the model that the two phenotypes are causally related (Conscience and Fischer, *Differentiation,* in press).
3. The lesion leading to factor-independent proliferation in the malignant cells seems to be located at the level of signal transduction between the IL-3 receptor and the cellular and nuclear targets of hormone action; it could involve an altered protein kinase C and/or phosphatidyl-inositol-diphosphate metabolism (Mazurek and Conscience, manuscript in preparation).
4. Expression of the c-*mic* and c-*fos* proto-oncogenes is IL-3 dependent in the non-malignant cells and has become constitutive in the transformed cells, thus indicating an essential role for these two genes in the regulation of cell proliferation, survival and differentiation in the basophil/mast cell lineage and, possibly, in other IL-3 regulated hemopoietic lineages (Conscience and Martin, manuscript in preparation).
5. Monoclonal antibodies directed against the transformed, as well as the non-transformed cell lines have been isolated; all of them cross-react extensively with both cell types, in support of the idea that the transformation event was not accompanied by a major shift in differentiated states. (Conscience et al., manuscript in preparation).

References

Ball, P.E., Conroy, M.C., Heusser, C.H., Davis, J.M., Conscience, J.-F. 1983: Spontaneous, in vitro, malignant transformation of a basophil/mast cell line. Differentiation, Vol. 24, 1983, 74–78.

Beug, H., Palmieri, S., Freudenstein, C., Zentgraf, H., Graf, T. 1982: Hormone-dependent terminal differentiation in vitro of chicken erythroleukemia cells transformed by *ts* mutants of avian erythroblastosis virus. Cell, Vol. 28, 1982, 907–919.

Conscience, J.-F., Meier, W. 1980: Coordinate expression of erythroid marker enzymes during dimethylsulfoxide-induced differentiation of Friend erythroleukemia cells. Experimental Cell Research, Vol. 125, 1980, 111–119.

Conscience, J.F., Meier, W. 1980: Coordinate expression of erythroid markers in differentiating Friend erythroleukaemic cells. In: Jimenez de Asua, L., Shields, R., Levi-Montalcini, R., Iacobelli, S.: Control Mechanisms In Animal Cells. Raven Press, New York.

Conscience, J.-F., Deslex, S., Fischer, F. 1982: Newly isolated Friend cell lines are blocked at the same stage of erythroid differentiation as established clones. Differentiation, Vol. 22, 1982, 100–105.

Davis, J.M., Kubler, A.-M., Conscience, J.-F. 1983: MBM-1, a differentiation marker of mouse hemopoietic cells defined by a rat monoclonal antibody. Experimental Hematology, Vol. 11, 1983, 332–340.

Denton, M.J., Spencer, N., Arnstein, H.R.V., 1975: Biochemical and enzymatic changes during erythrocyte differentiation. Biochemical Journal, Vol. 146, 1975, 205–211.

Deslex, S. 1982: Isolement et caractérisation de nouvelles lignées transformées par le virus de Friend. Thèse de doctorat (3ème cycle), Université Louis-Pasteur, Strasbourg.

Galli, S.J., Dvorak, A.M., Marcum, J.A., Ishizaka, T., Nabel, G., Der Simonian, H., Pyne, K., Goldin, J.M., Rosenberg, R.D., Cantor, H., Dvorak, H.F. 1982: Mast cell clones: A model for the analysis of cellular maturation. Journal of Cell Biology, Vol. 95, 1982, 435–444.

Geiduschek, J.B., Singer, S.J. 1979: Molecular changes in the membrane of mouse erythroid cells accompanying differentiation. Cell, Vol. 16, 1979, 149–163.

Greaves, M.F. 1982: "Taget" cells, cellular phenotypes, and lineage fidelity in human leukaemia. Journal of Cellular Physiology, Supplement 1, 1982, 113–125.
Holley, R.W. 1972: A unifying hypothesis concerning the nature of malignant growth. Proceedings of the National Academy of Sciences of the United States of America, Vol. 69, 1972, 2840–2841.
Ibsen, K.H., Fishman, W.H. 1979: Developmental gene expression in cancer. Biochimica Biophysica Acta, Vol. 560, 1979, 243–280.
Kennett, R.H., McKearn, T.J., Bechtol, K.B. 1980: Monoclonal Antibodies. Plenum Press, New York and London.
Marks, P.A., Rifkind, R.A. 1978: Erythroleukemic differentiation. Annual Reviews of Biochemistry, Vol. 47, 1978, 419–448.
Matsuda, H., Kitamura, Y., Sonoda, D., Imori, T. 1981: Precursors of mast cells fixed in the skin of mice. Journal of Cellular Physiology, Vol. 108, 1981, 409–415.
Metcalf, D. 1977: Hemopoietic colonies. Springer Verlag, Berlin.
Moore, M.A.S., Sheridan, A.P. 1982: The role of proliferation and maturation factors in myeloid leukemia. Progress in Cancer Research and Therapy, Vol. 23, 1982, 361–376.
Ralph, P., Nakoinz, I., Raschke, W.C. 1982: Induction of differentiated functions coupled with growth inhibition in lymphocyte and macrophage tumor cell lines. Progress in Cancer Research and Therapy, Vol. 23, 1982, 245–255.
Sachs, L. 1978: Control of normal cell differentiation and the phenotypic reversion of malignancy in myeloid leukemia. Nature, Vol. 274, 1978, 535–539.
Sachs, L. 1980: Constitutive uncoupling of pathways of gene expression that control growth and differentiation in myeloid leukemia: A model for the origin and progression of malignancy. Proceedings of the National Academy of Sciences of the United States of America, Vol. 77, 1980, 6125–6156.
Setchenska, M.S., Arnstein, H.R.V. 1978: Changes in the lactate dehydrogenase isoenzyme pattern during differentiation of rabbit bone marrow erythroid cells. Biochemical Journal, Vol. 170, 1978, 193–201.
Till, J.E., McCulloch, E.A. 1980: Hemopoietic stem cell differentiation. Biochimica Biophysica Acta, Vol. 605, 1980, 431–459.
Till, J.E. 1982: Stem cells in differentiation and neoplasia. Journal of Cellular Physiology, Supplement 1, 1982, 3–11.
Volloch, V., Housman, D. 1982: Terminal differentiation of murine erythroleukemia cells: Physical stabilization of end-stage cells. Journal of Cell Biology, Vol. 93, 1982, 390–394.
Warner, N.L., Cheney, R.K., Lanier, L.L., Daley, M., Walker, E. 1982: Differentiation heterogeneity in murine hematopoietic tumors. Progress in Cancer Research and Therapy, Vol. 23, 1982, 223–242.
Wigley, C.B. 1975: Differentiated cells in vitro. Differentiation, Vol. 4, 1975, 25–55.

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Developmental regulation of tumor autonomy in plants

Frederick Meins, Jr.

Summary

Neoplastic diseases of plants have several proximal causes; for example, genetic transformation by plasmids of bacterial origin; transformation by double stranded RNA viruses; and, the combination of foreign genomes in interspecific plant hybrids. Regardless of proximal cause, autonomous growth in these diseases appears to result from the inappropriate production of growth factors by the tumor cells. There is evidence that genetic changes are neither necessary nor sufficient for expression of the tumor state in plants: autonomously growing cells can arise as a consequence of epigenetic changes. Tumor cells that contain foreign genes necessary for autonomous growth can differentiate, participate in organogenesis, and exhibit normal growth regulation. This suggests that even when transformation results from a genetic lesion, expression of the tumor state is ultimately regulated by epigenetic mechanisms of the type operating in normal development.

Introduction

As a result of neoplastic transformation, cells acquire the capacity for autonomous growth, which is then inherited by individual tumor cells. Plant neoplastic diseases provide experimental systems well suited for studying the transformation process since the physiological basis for autonomous growth is known and it is possible to establish the developmental and genetic potentialities of the tumor cell by regeneration of complete plants.

In this article I will briefly review the biology of plant tumors using crown gall disease as an example and then provide evidence to support the hypothesis that tumor transformation in plants is a form of inappropriate cell differentiation which is precisely regulated by the same types of mechanisms that operate in normal development.

Tumor inception in crown-gall

Crown-gall tumors result when cells conditioned by wounding from a wide variety of dicotyledonous plant species and certain gymnosperms are exposed to a tumor inducing principle (TIP) elaborated by virulent strains of *Agrobacterium tumefaciens*. The tumors which form at the site of inoculation are true tumors since the cells continue to express their neoplastic character in culture and when grafted onto the host plant in the absence of the inciting bacterium. The type of tumor obtained depends on the plant host, the site of inoculation, and the strain of bacterium used (Braun 1978). For example, *Kalanchoë* or tobacco plants inoculated with the B6 strain of bacterium form large, unorganized tumors that produce the opine octopine, whereas plants inoculated with the T37 strain form complex, highly organized tumors, known as teratomas, that produce another opine, nopaline. Both the pattern of development and type of opine made persist in cloned cell lines serially transferred in culture, indicating that these traits are inherited at the cellular level.

There are several lines of evidence indicating that TIP is a DNA sequence derived from a large, tumor-inducing plasmid (Ti) present in virulent strains of the crown-gall bacterium. First, during tumor inception a portion of this plasmid, T-DNA, is transferred to the host cell (Chilton *et al.* 1977) where it is integrated into one or more sites in the nuclear DNA (Yadav *et al.* 1980). Second, the type of opine produced by the tumor cell is specified by T-DNA (Bomhoff *et al.* 1976), which, in the case of octopine plasmids, for

example, codes for the key enzyme in the synthesis of octopine (Schröder *et al.* 1981; Murai and Kemp 1982). Third, mutations at specific sites in the T-DNA affect the growth and development of tumors elicited with the mutant strains of plasmid (Hooykaas *et al.* 1982; Garfinkel *et al.* 1981). Finally, reversion of tumor cells is accompanied by the loss of all, or a major portion, of the T-DNA (Yang *et al.* 1980; Yang and Simpson 1981). Thus it appears that the maintenance of the tumor state in crown-gall depends on the presence of Ti-plasmid-DNA sequences that are transferred from the bacterium to the plant cell during tumor inception.

Growth-promoting substances and tumor autonomy

Comparison of the nutritional requirements of normal and tumor cells in culture provides strong evidence that the autonomous growth of plant tumors involves an alteration in the capacity of the cell to produce growth factors. Several growth factors are required for proliferation of higher plant cells in culture. The most important ones are the auxins, which promote DNA synthesis, mitosis and cell enlargement, and cell-division factors, such as the cytokinins, which promote cell division. For exemple, pith cells of tobacco exhibit an absolute requirement for exogenous auxin and cytokinin for continuous growth on an otherwise complete medium (Jablonski and Skoog 1954). As a result of transformation, these cells acquire the capacity to grow without the added factors and produce auxin and cytokinin in quantities sufficient to support the growth of normal cells (Braun 1958). Similar changes, although less well-documented, are also found in tumors arising in tumor-prone genetic hybrids (Bayer 1982) and in wound tumor disease, which is caused by a double-stranded RNA virus (Black 1982). These findings lead to the important conclusion that, regardless of the proximal cause for transformation, autonomous growth results from changes in the production of substances necessary for the proliferation of normal cells (Braun 1958; Meins 1974).

The details of how production of growth factors is regulated in tumor cells and the relationship between growth and production of these factors is not known. The observation that crown-gall tumors on plants mimic the application of auxin has lead to the hypothesis that tumor autonomy results from the overproduction of auxin (Link and Eggers 1941). This seems unlikely. Tumor tissues *in planta* and in culture contain auxin at concentrations in the range found in rapidly growing normal tissues of the same plant species (Weiler and Spanier 1981; Pengelly and Meins 1981). There is growing evidence that different genes in the T-DNA affect auxin and cytokinin production by the tumor cell (Garfinkel *et al.* 1981; Hooykaas *et al.* 1982; Morris *et al.* 1982). It is not known, however, whether these genes code for enzymes needed for the synthesis of these factors or act indirectly by altering the expression of host genes for these enzymes.

Developmental regulation of growth autonomy

Insight into the relative contribution of foreign and host genes in the regulation of growth autonomy is provided by the observation that heritable changes in auxin and cytokinin requirement similar to those encountered in transformation also occur in normal development. For example, leaf cells of tobacco require exogenous cytokinin for rapid growth in culture, whereas stem-cortex cells do not (Meins and Lutz 1979). The fact that both states are inherited by individual cells implies that heritable changes in cytokinin requirement occurred sometime in the development of the two tissue types. Direct evidence that this can result in autonomous growth comes from studies of habituation (Meins 1982a).

Tissues from a variety of plant species in culture sometimes lose their requirement for exogenous auxin, or for both auxin and cytokinin. This process, known as habituation, is progressive and generates autonomously proliferating cells, which, in some cases, form transplantable tumors when grafted onto the host plant. Therefore, it appears that habituation is a form of spontaneous neoplastic transformation that occurs in culture.

In spite of their extreme phenotypic stability when serially propagated in culture, cytokinin-habituated cells can be induced to lose

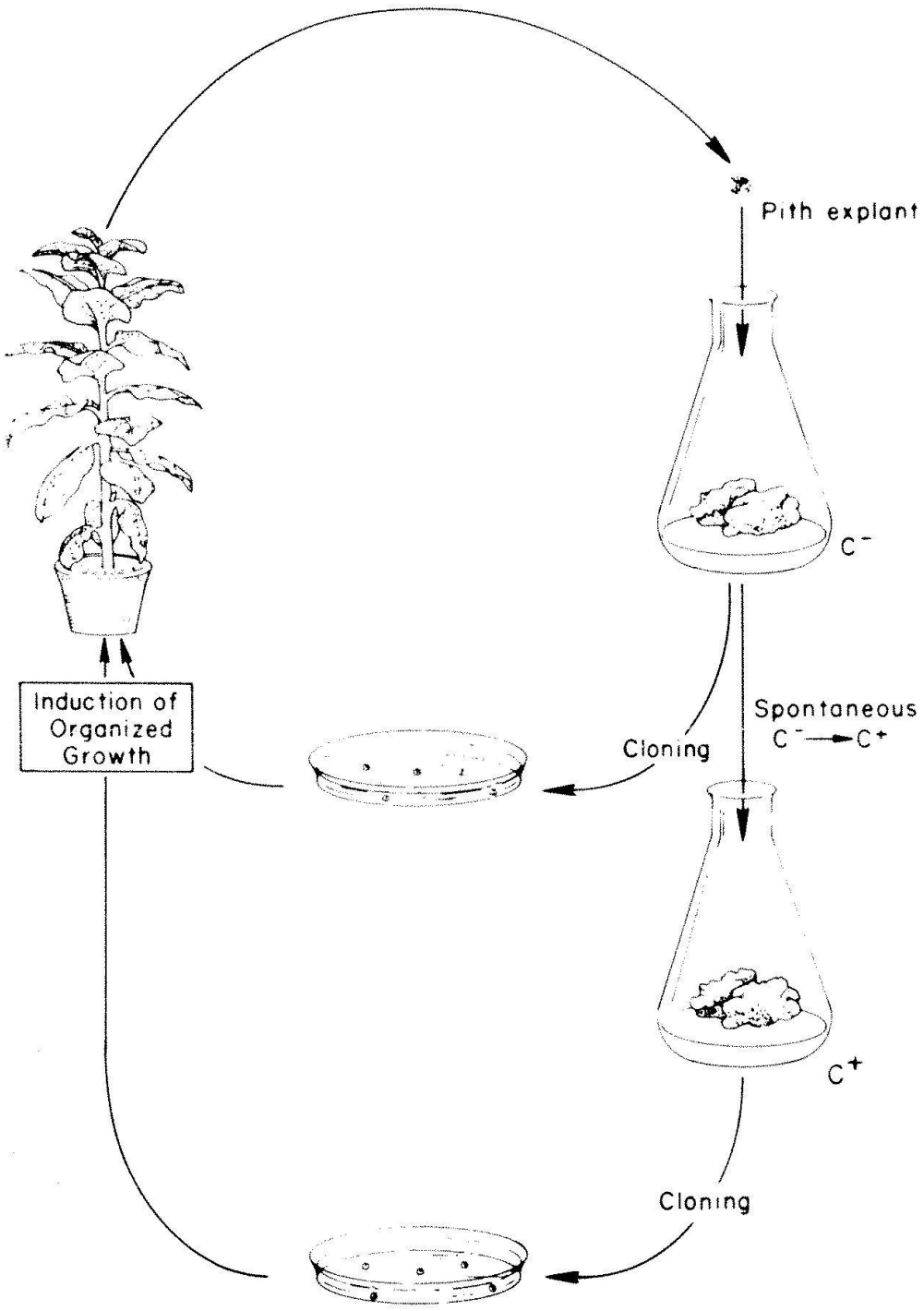

Fig. 1. Plant regeneration experiment showing that the cytokinin-habituated phenotype (C^+) reverts to the non-habituated phenotype (C^-) when cloned tobacco cells of pith origin are induced to form complete plants. From Meins 1974.

their autonomous character. Cloned lines of habituated tobacco cells, which are of pith origin, regularly form normal-looking, fertile tobacco plants when placed on a differentiation-inducing medium. Pith tissues from the regenerated plants exhibit the normal, non-habituated phenotype in culture (fig. 1). Results of this type obtained with many cloned lines and detailed measurements of the rates at which habituation is induced and reversed in culture indicate that habituation involves heritable, potentially reversible, epigenetic changes rather than classical mutations (Meins 1982a). Conversion of cells to the cytokinin-autotrophic state is a key event in both tumor transformation and habituation, which has an epigenetic basis. Therefore, neither somatic mutation nor the introduction of foreign genes need be invoked to account for the stability of the tumor state.

Functions essential for autonomous growth can also be switched on and off in tumor cells containing foreign genes (Braun 1959;

Braun and Wood 1976; Turgeon *et al.* 1976). When cloned, crown-gall teratoma tissues of tobacco are grafted onto the cut stem of tobacco plants from which the auxillary buds have been removed, they give rise to shoots which gradually become more normal in appearance. Some of these shoots eventually flower and set fertile seed. These seeds develop into normal tobacco plants, which have completely lost their tumorous character and, as expected, no longer contain readily detectable T-DNA sequences (Yang *et al.* 1980). Reversion with the loss of Ti-plasmid genes also occurs spontaneously in cultured teratoma tissues, but at a low incidence (Yang and Simpson 1981; Meins 1982a).

Of particular interest, however, are those cases in which tumor cells mimic the normal state but still retain T-DNA sequences. Leaves near the top of teratoma-derived shoots are perfectly normal in gross morphology, physiology and histology. Nevertheless these leaves still produce nopaline (Wood *et al.* 1978), contain T-DNA (Yang *et al.* 1980), and, when placed in culture on a basal medium that does not support the growth of normal leaf tissue, give rise to typical teratoma tissues. It may be argued that the teratoma-derived shoots consist predominantly of revertant cells and that teratomatous tissues arise from a few tumor cells that still retain T-DNA. There is strong evidence against this. Protoplasts from highly differentiated mesophyll cells isolated from leaves on teratoma-derived shoots regularly form teratomatous clones when cultured (Binns *et al.* 1981). Similar results are obtained with single, highly specialized, epidermal hair cells (Huff and Turgeon 1981). Thus, teratoma-derived shoots consist of tumor cells that differentiate, participate in organogenesis, and exhibit normal growth regulation while retaining their tumorous character in a covert form.

Suppression of the tumorous state also occurs in plants regenerated from tumors of genetic hybrids. Carlson *et al.* (1972) have fused protoplasts from two species of *Nicotiana* that give tumor-prone hybrid plants. The dikaryotic clones which result, like crown-gall tissues, do not require auxin and cytokinin for growth in culture. Plants regenerated from these clones are tumor prone; when wounded, typical teratomas arise at the wound site. This shows that cells genetically predisposed to grow autonomously do not express their tumor character in the intact plant, even though the cells retain this potential. Finally, there is a mutation of tobacco in which leaf cells exhibit the cytokinin-habituated phenotype in culture (Meins, 1982c). Plants regenerated from these cells develop normally and have normal appearing leaves, but the leaf tissue still expresses its autonomous character when placed in culture. Therefore, autonomous growth whether resulting from the incorporation of plasmid genes, mutation, or the hybridization of dissimilar genomes is phenotypically suppressed when plants or shoots are regenerated from the altered cells.

Conclusion

There is good evidence that the autonomous growth of plant tumor cells involves the inappropriate production of growth substances such as auxin and cytokinin. What is not clear, at present, is the molecular basis for these changes. It is still not known whether the primary lesion in different tumor diseases is the same or not, whether growth-factor requirement is regulated at the level of growth-factor production or growth-factor degradation, and how foreign genes regulate these processes.

Genetic events such as the integration of foreign genes, interspecific hybridization and somatic mutation appear to be neither necessary nor sufficient for the expression of growth autonomy. Autonomous tumor cells can arise by epigenetic changes that do not seem to involve permanent genetic changes. Studies of teratoma derived shoots and genetic hybrid tumors show that expression of the tumor state can be blocked even when transformation results from a genetic lesion. The picture that emerges is that transformation is a form of abnormal differentiation subject to regulation by the same types of epigenetic mechanisms involved in normal development.

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Updating note

Since this article was written in 1982, direct evidence has been obtained that genes in the T-DNA code for enzymes important in auxin and cytokinin production (Barry *et al.*, 1984; Inze *et al.*, 1983; Schroeder *et al.*, 1984). Comparison of the cytokinin and auxin contents of habituated and crown gall transformed cells has provided further support for the hypothesis that plant cells contain genes with functions similar to the oncogenic genes in the T-DNA (Pengelly and Meins, 1983; Hansen *et al.*, in press). An excellent review of the problem of tumor autonomy in plants has recently appeared (Binns, 1983).

References

Barry, G.F., Rogers, S.G., Fraley, R.T., Brand, L. 1984. Identification of a cloned cytokinin biosynthetic gene. Proc. Natl. Acad. Sci. USA., 81, 4776–4780.

Bayer, M. 1982: Genetic tumors: Physiological aspects of tumor formation in interspecies hybrids. In: Kahl, G., Schell, J.S.: Molecular biology of plant tumors. Academic, New York and London.

Binns, A., Wood, H.N., Braun, A.C. 1981: Suppression of the tumorous state in Crown gall teratomas of tobacco. A clonal analysis. Differentiation 19, 97–102.

Binns, A.N. 1983. The biology and molecular biology of plant cells infected by *Agrobacterium tumefaciens*. In: Miflin, B.J.: Oxford Surveys of Plant Molecular and Cell Biology, pp. 133–160, Clarendon, Oxford.

Black, L.M. 1982: Wound tumor disease. In: Kahl, G., Schell, J.S.: Molecular biology of plant tumors. Academic, New York and London.

Bomhoff, G., Klapwijk, P.M., Kester, H.C.M., Schilperoort, R.A., Hernalsteens, J.P., Schell, J. 1976: Octopine and nopaline synthesis genetically controlled by a plasmid of *Agrobacterium tumefaciens*. Molec. and Gen. Genetics 145, 177–181.

Braun, A.C. 1959: A demonstration of the recovery of the crown-gall tumor cell with the use of complex tumors of single-cell origin. Proc. Natl. Acad. Sci. U.S.A. 45, 932–938.

Braun, A.C. 1978: Plant tumors. Biochim. Biophys. acta 516, 167–191.

Braun, A.C., Wood, H.N. 1976: Suppression of the neoplastic state with the acquisition of specialized functions in cells, tissues, and organs of crown-gall teratomas of tobacco. Proc. Natl. Acad. Sci. U.S.A. 73, 469–500.

Carlson, P.S., Smith, H.H., Dearing, R.D. 1972: Parasexual interspecific plant hybridization. Proc. Natl. Acad. Sci. U.S.A. 69, 2292–2294.

Chilton, M.-D., Drummond, M.H., Merlo, D.J., Sciaky, D., Montoya, A.L., Gordon, M.P., Nestor, E.W. 1977: Stable incorporation of plasmid DNA into higher plant cells: The molecular basis of Crown gall tumorigenesis. Cell. 11, 263–271.

Garfinkel, D.J., Simpson, R.B., Ream, L.W., White, F.F., Gordon, M.P., Nester, E.W. 1981: Genetic analysis of crown gall: Fine structure map of the T-DNA by site-directed mutagenesis. Cell. 27, 143–153.

Hansen, C.E., Meins, F., Jr., Milani, A. Clonal and physiological variation in the cytokinin content of tobacco-cell lines differing in cytokinin requirement and capacity for neoplastic growth. Differentiation, in press.

Hooykaas, P.J.J., Ooms, G., Schilperoot, R.A. 1982: Tumors induced by different strains of *Agrobacterium tumefaciens*. In: Kahl, G., Schell, J.S.: Molecular biology of plant tumors. Academic, New York and London.

Huff, M., Turgeon, R. 1981: Neoplastic potential of trichomes isolated from tobacco crown gall teratomas. Differentiation. 19, 93.

Inze, D., Follin, A., Van Lijsebettens, M., Simeons, C., Genetello, C., Van Montagu, M., Schell, J. 1984. Genetic analysis of the individual T-DNA genes of *Agrobacterium tumefaciens;* further evidence that two genes are involved in indole-3-acetic acid synthesis. Molec. Gen. Genet., 194, 265–274.

Jablonski, J.R., Skoog, F. 1954: Cell enlargement and cell division in excised tobacco pith tissue. Physiol. Plant. 7, 16–24.

Link, G.K.K., Eggers, V. 1941: Hyperauxiny in crown gall of tomato. Bot. Gaz. 103, 87–106.

Meins, F., Jr. 1974: Mechanisms underlying tumor transformation and tumor reversal in crown-gall, a neoplastic disease of higher plants. Symp. Soc. Devel. Biol. 32, 23–39.

Meins, F., Jr. 1982a: Habituation of cultured plant cells. In: Kahl, G., Schell, J.S.: Molecular biology of plant tumors. Academic, New York and London.

Meins, F., Jr. 1982b: Tumor reversal in plants. Prog. Clinical Biol. Rsch. 85B, 567–576.

Meins, F., Jr. 1982c: The nature of the cellular heritable change in cytokinin habituation. In: Earle, E., Demarly, Y.: Variability in plants regenerated from tissue culture. Praeger, New York.

Meins, F., Jr., Lutz, J. 1979: Tissue-specific variation in the cytokinin habituation of cultured tobacco cells. Differentiation. 15, 1–6.

Morris, R.O., Akiyoshi, D.E., MacDonald, E.M.S., Morris, J.W., Regier, D.A., Zaerr, J.B. 1982: Cytokinin metabolism in relation to tumor induction by *Agrobacterium tumefaciens*. In: Wareing, P.F.: Plant growth substances 1982. Academic Press, London and New York.

Murai, N., Kemp, J. 1982: Octopine synthase mRNA isolated from sunflower crown gall callus is homologous to the Ti plasmid of *Agrobacterium tumefaciens*. Proc. Natl. Acad. Sci. U.S.A. 79, 86–90.

Pengelly, W.L., Meins, F., Jr. 1981: The relationship of indole-3-acetic acid content and growth of crown-gall tumor tissues of tobacco in culture. Differentiation. 21, 27–31.

Pengelly, W.L., Meins, F., Jr. 1983. Growth, auxin requirement, and indole-3-acetic acid content of cultured crown-gall and habituated tissues of tobacco. Differentiation, 25, 101–105.

Schröder, J., Schröder, G., Huismann, H., Schilperoort, R.A., Schell, J. 1981: The mRNA for lysopine dehydrogenase in plant tumor cells is complementary to a Ti-plasmid fragment. FEBS Letters 129, 166–168.

Schroeder, G., Waffenschmidt, S., Weiler, E.W., Schroeder, J. 1984. The T-region of Ti plasmid codes for an enzyme synthesizing indole-3-acetic acid. Eur. J. Biochem., 138, 387–391.

Turgeon, R., Wood, H.N., Braun, A.C. 1976: Studies on the recovery of Crown gall tumor cells. Proc. Natl. Acad. Sci. U.S.A. 73, 3562–3564.

Weiler, E.W., Spanier, K. 1981: Phytohormones in the formation of crown gall tumors. Planta 153, 326–337.

Wood, H.N., Binns, A.N., Braun, A.C. 1978: Differential expression of oncogenicity and nopoline synthesis in intact leaves derived from crown gall teratomas of tobacco. Differentiation. 11, 175–180.

Yadav, N.S., Postle, K., Saiki, R.K., Thomashaw, M.F., Chilton, M.-D. 1980: T-DNA of a crown gall teratoma is covalently joined to host plant DNA. Nature. 287, 458–461.

Yang, F.-M., Montoya, A., Merlo, D.J., Drummond, M.H., Chilton, M.-D., Nester, E.W., Gordon, M.P. 1980: Foreign DNA sequences in crown gall teratomas and their fate during the loss of the tumorous trait. Molec. Gen. Genetics. 177, 707–714.

Yang, F.-M., Simpson, R. 1981: Revertant seedlings from crown gall tumors retain a portion of the bacterial T-DNA sequences. Proc. Natl. Acad. Sci. U.S.A. 78, 4151–4155.

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Detection of human carcinoma by immunoscintigraphy using monoclonal anti-CEA antibodies

Michel Forni, Jurg Ritschard, Franz Buchegger, Charles Haskell, Stephan Carrel and Jean-Pierre Mach

Early experimental results

Research on tumour localization of radiolabeled antibodies was initiated almost 30 years ago by Pressman (1) and Bale (2), who showed that labeled antibodies against Wagner osteosarcoma or Walker carcinoma cells were concentrated *in vivo* by these tumors.
In 1974, we introduced into this field the model of nude mice bearing grafts of human colon carcinoma and the use of affinity purified antibodies against carcinoembryonic antigen (CEA) (3). We showed that purified ^{131}I-labeled goat anti-CEA antibodies could reach up to a 9 times higher concentration in the tumor than in the liver, while the concentration of control normal IgG in the tumor was never higher than 2.3 times that in the liver. We observed, however, great variations in the degree of specific tumor localisation by the same preparation of labeled antibodies, when colon carcinoma grafts de-

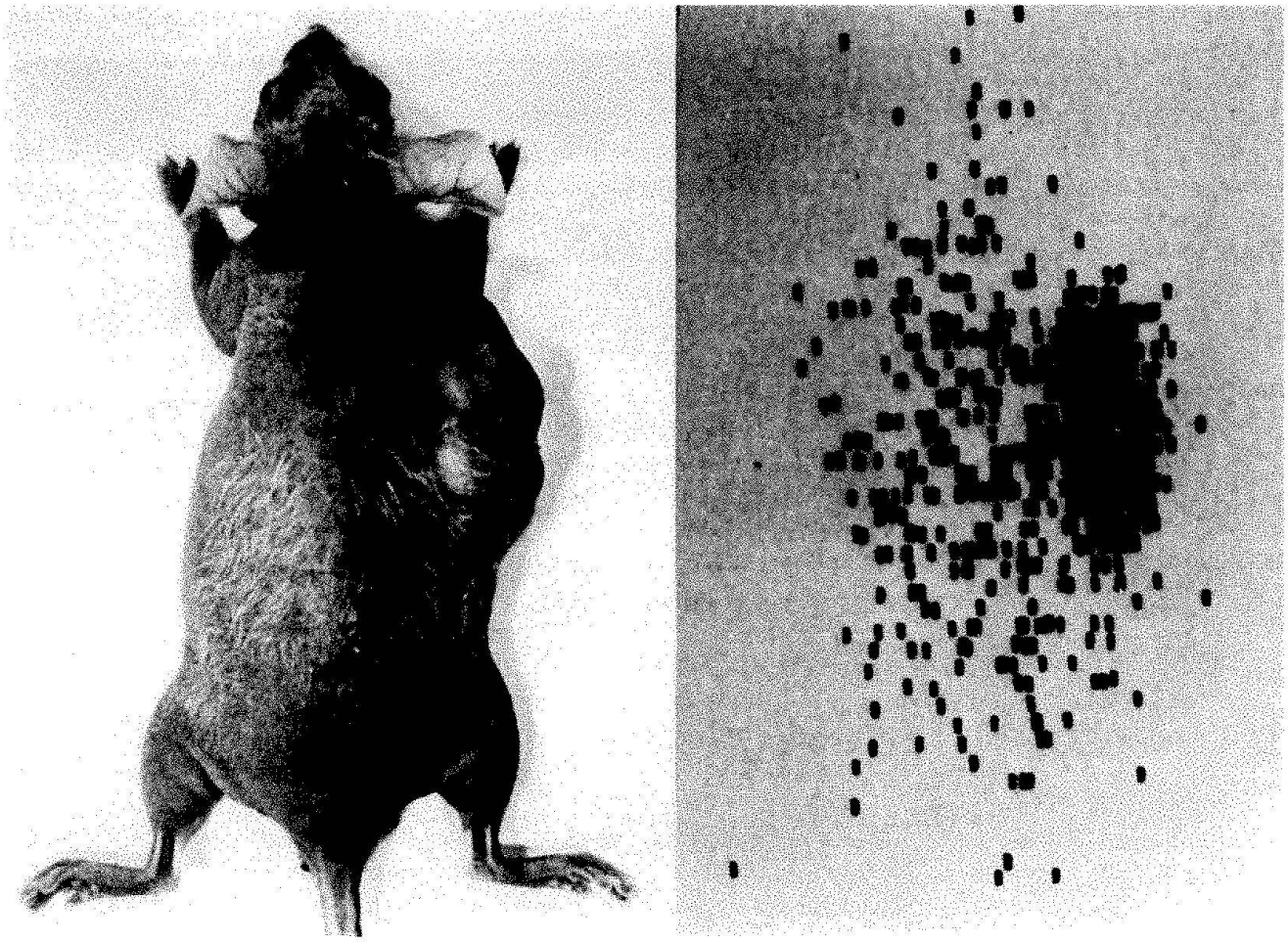

Fig. 1. Scanning of a nude mouse which received an injection of ^{131}I-labeled anti-CEA antibodies. A. Nude mouse bearing a xenograft of human colon carcinoma shown in the scanning position. B. The total body scan from the same mouse obtained 3 days after injection of 2 ug of ^{131}I-labeled anti-CEA antibodies (dose of radioactivity injected = 16 uCi). (Reproduced with the permission of *Nature*) (3).

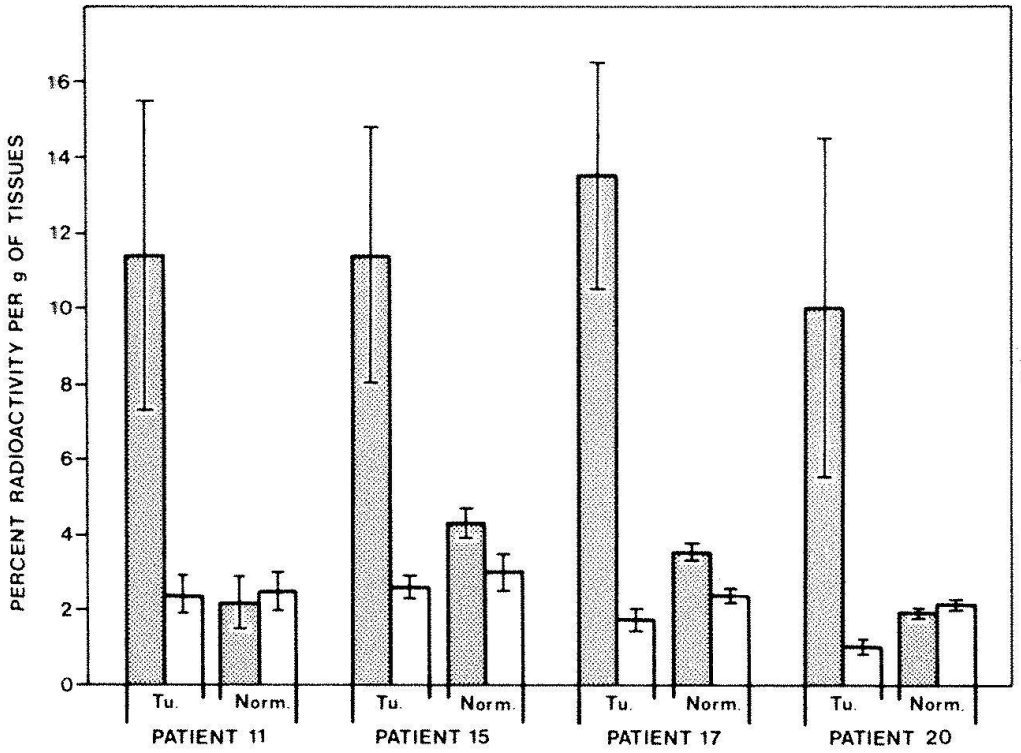

Fig. 2. Specific tumor uptake of anti-CEA antibodies in patients with colon carcinoma. The shaded areas show the relative concentration of ^{131}I-labeled control normal goat IgG, in both tumor (Tu.) and normal mucosa (Norm.) from four patients who received simultaneous injections of both labeled proteins 3–8 days before surgery. The vertical solid lines show the standard deviation of the results obtained in individual tissue fragments. The radioactivity of each isotope present in each fragment was measured in a dual channel gamma counter. (Reproduced with the permission of *N. Engl. J. Med.)* (8).

rived from different donors were tested. This is probably due to the fact that human tumors keep their initial histologic properties and degree of differentiation after transplantation into nude mice and these two factors appear to affect the ease with which circulating antibodies gain access to the CEA present in tumors. The detection of ^{131}I-labeled antibodies in tumors by external scanning also gave variable results. With colon carcinoma grafts from certain donors we obtained scans with good tumor localisation, such as the one presented in figure 1, whereas with colon carcinoma grafts from other donors the antibody uptake was not sufficient to give satisfactory scanning images. In this context we think that results in the nude mouse model are a good reflection of the clinical reality observed in patients.

Independently, Goldenberg *et al.* (4) showed specific tumor localisation and detection by external scanning with ^{131}I-labeled IgG fractions of anti-CEA serum, using two human carcinomas which had been serially transplanted into hamsters for several years. Using the same experimental model Hoffer *et al.* (5) also demonstrated tumor localisation with radiolabeled IgG anti-CEA by external scanning.

Clinical results with polyclonal anti-CEA antibodies

The first detection of carcinoma in patients obtained by external scanning following injection of purified ^{131}I-labeled anti-CEA antibodies was reported by Goldenberg *et al.* (6,7). They claimed that almost all the CEA producing tumors could be detected by this method and that there was no false positive results. However, our experience, using highly purified goat anti-CEA antibodies and the same blood pool subtraction technology as Goldenberg was that only 42% of CEA producing tumors (22 out of 53 tested) could be detected by this method (8–9). Furthermore, we found that in several patients the labeled anti-CEA antibodies localized non-specifically in the reticuloendothelium. Despite the use of the subtraction technology, these non-specific uptakes were difficult to differentiate from the specific uptakes corresponding to the tumors. This discrepancy of results is unlikely to be due to a difference in the quality of the anti-CEA used since we showed by direct measurement of the radioactivity in tumors resected after injection, that our antibody was capable of excellent tumor localization (8) (fig. 2). Furthermore, in a few patients scheduled for

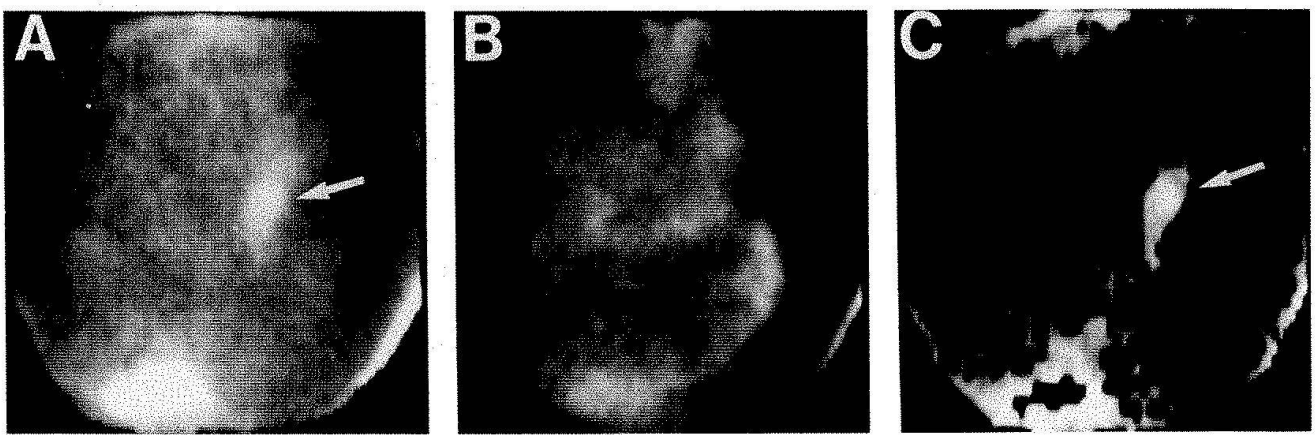

Fig. 3. Localization of carcinoma by external photoscanning after injection of radiolabeled $F(ab')_2$ fragments of monoclonal antibodies against CEA. Anterior left lateral photoscans of the whole abdomen and pelvis of a patient who received an injection of ^{131}I-labeled $F(ab')_2$ fragments of Mab 35, 48 hrs before scanning. The patient had a metastasis of a carcinoma of the ovary, located deep in the abdomen below the umbilicus. Panel A shows the total ^{131}I radioactivity, panel B the ^{99}Tc radioactivity due to injection of ^{99}Tc-labeled HSA and free $^{99}TcO_4$, 15 min before scanning. Panel C shows the remaining ^{131}I radioactivity after subtraction of ^{99}Tc radioactivity. In panel A and C the arrow indicate the radioactive spot corresponding to the tumor.

tumor resection, we injected simultaneously 1 mg of goat anti-CEA antibodies labeled with 1 mCi of ^{131}I and 1 mg of control normal goat IgG labeled with 0.2 mCi of ^{125}I. By this paired labeled method adapted to the patient situation, we could demonstrate that the antibody uptake was 4 times higher than that of control normal IgG (8) (fig. 2).

These results were very encouraging in terms of specificity of tumor localization. However, the direct measurement of radioactivity in tumors also showed that only 0.05-0.2% of the injected radioactivity (0.5-2 μCi out of 1000 μCi) were recovered in the resected tumors 3–8 days after injection (8). This information is essential if one is considering the use of ^{131}I labeled antibody for therapy (10).

Monoclonal anti-CEA antibody used in photoscanning

The obvious advantage of monoclonal antibodies (Mabs) are their homogeneity and their specificity for the immunizing antigen. Another advantage of Mabs is that they each react with a single antigenic determinant and thus should not be able to form large immune complexes with the antigen (provided that the antigenic determinant is not repetitive).

The first Mab anti-CEA used for immunoscintigraphy in patients was Mab 23 (11). Its production and characteristics have been described (12). Mab 23 was given intravenously to 26 patients with large bowel carcinomas and 2 patients with pancreatic carcinomas. Each patient received 0.3 mg of purified Mab labeled with 1–1.5 mCi of ^{131}I. The patient's premedication included lugol 5 percent iodine solution, promethazine and prednisolone, as previously described (8,9). The patients had no personal history of allergy. They were also tested with an intracutaneous injection of normal mouse IgG and found to have no hypersensitivity against this protein. None of the patients showed any sign of discomfort during or after the injection of labeled mouse antibodies. The patients were studied by external photoscanning 24, 36, 48 and 72 h after injection. An Elscint large-field camera with an LFC9 high-energy parallel-hole collimator was used. In 14 of the 28 patients (50%) a radioactive spot corresponding to the tumor was detected 36–48 h after injection. In 6 patients the scans were doubtful and in the remaining 8 patients they were entirely negative (11).

In order to improve these results we produced a series of 26 new hybrids secreting anti-CEA antibodies. Three of them were selected by criteria of high affinity for CEA of the antibody produced (13). The new selected Mabs designated 202, 35 and 192 were purified and tested for the detection of human carcinoma grafted in nude mice both in the form of intact Mab and in the form of

$F(ab')_2$ and Fab fragments. The results showed that the fragment of Mabs were markedly superior to the intact Mab for the detection of human carcinoma in the nude mouse model (14).

One of the positive scanning studies obtained recently with the $F(ab')_2$ fragment of Mab 35 is illustrated in figure 3. The patient was an 87 years old female with a retroperitoneal metastasis of a carcinoma of the ovary. The tumor was palpable below the umbilicus and found by CAT SCAN to consist of a solid masse of 13x9 cm diameter. Figure 3 shows the anterior-lateral photoscans (camera to the left of the patient) of the abdomen and pelvis, taken 48 hrs after injection of 0.3 mg of Mab 35 $F(ab')_2$ labeled with 1.5 mCi of ^{131}I (55.5 MBq). Panel A shows the total ^{131}I radioactivity; one sees a diffuse radioactive spot in the center right of the scan (arrow) corresponding to the tumor. Abundant radioactivity is also present in the lower left part of the scan corresponding to the urinary bladder which was not properly emptied. Panel B shows the ^{99m}Tc radioactivity due to the injection, 15 min before scanning, of 500 µCi (18.5 MBq) of ^{99m}Tc labeled human serum albumin and 500 µCi of free $^{99m}TCO^-_4$. There are diffuse spots of radioactivity in the stomach, spleen and bladder as well as in the left side of the abdomen but these spots do not correspond to the localization of the tumor. Panel C shows the ^{131}I radioactivity after computerized subtraction of the ^{99m}Tc radioactivity. Here, the major central radioactive uptake corresponding to the tumor is well contrasted. The urinary bladder remained positive despite the subtraction.

Tumor detection by tomoscintigraphy

Another way to improve tumor detection by immunoscintigraphy is the use of tomoscintigraphy. As we have seen, static photoscanning is limited in part by the presence of radiolabeled antibodies or free ^{131}I released from them, in the circulation, the reticuloendothelial system, the stomach, intestine and urinary bladder. Increased radioactivity in these compartments may give false positive results. Specific tumor sites may be masked by non-specific radioactivity. These problems cannot be entirely resolved by the presently available subtraction methods using ^{99m}Tc labeled HSA and free $^{99m}TcO^-_4$. Axial transverse tomoscintigraphy is a method initially developed by Kuhl and Edwards in 1973 (15) with the potential to resolve some of these problems. This method, also called single photon *emission* computerized tomography (SPECT), corresponds to the application of the tomographic technique used in *transmission* computerized axial tomography (CAT) to scintigraphic data. Mathematical techniques similar to those used in positron and X-ray tomographies allows the reconstruction of transverse sections as well as frontal, saggital or oblique sections of patients. In collaboration with Ch. Berche and J.-D. Lumbroso from the Institut Gustave Roussy in Villejuif, we have recently shown that tomoscintigraphy can improve the sensivity and specificity of tumor detection by radiolabeled anti-CEA Mabs (16). With this methods 15 out of 16 carcinoma tumor sites studied (including 10 colorectal carcinomas, 1 stomach, 1 pancreas and 4 medullary thyroid carcinomas) were detectable. These results are encouraging in term of sensitivity. However, it should be noted that numerous non-specific radioactive spots, sometimes as intense as the tumors were observed. Thus, the problem of non-specific accumulation of antibodies remains, but the three dimensions localization of radioactive spots by tomoscintigraphy, helped to discriminate specific tumor uptakes from the non-specific ones (16).

Antibody and control radioactivity in resected tumors

A few patients scheduled for tumor resection were injected simultaneously with 1 mCi of ^{131}I-labeled Mabs and 0.2 nCi of ^{125}I-labeled purified normal mouse IgG. The radioactivity of both isotopes was measured in the resected tumors and adjacent normal tissues and the specificity of tumor locatization studied by differential radioactive analysis, as shown in table 1. One sees that the factors of radioactivity uptake (indicated in parentheses) range from 2.7 to 7.4 for Mab 23 when one compares tumor with normal mucosa. They are even higher when one com-

Table 1. Specificity of tumor localisation of monoclonal anti-CEA antibodies as compared to normal IgG*

Case No. Tumour site	Tumour weight	Material injected	Tot.nCi Tumour	nCi/g Tumour	nCi/g N.mucosa	Tumour** N.mucosa	nCi/g N.serosa	Tumour** N.serosa	nCi/ml serum	Tumour** serum
63) Tr.colon op.d.6; CEA = 1.2	15 g	Mab 23 ^{131}I	425	28.3	10.3	(2.7)** 3.5***	6.6	(4.3) 4.8	30.3	(0.9) 5.4
		N.-IgG ^{125}I	45	3.0	3.8	(0.8)	3.3	(0.9)	17.5	(0.17)
67) Left colon op.d.8; CEA = 1.1	16 g	Mab 23 ^{131}I	196	12.3	4.6	(2.7)** 2.2***	2.5	(4.9) 3.0	17.7	(0.7) 3.3
		N.-IgG ^{125}I	50	3.1	2.5	(1.2)	1.9	(1.6)	14.8	(0.2)
73) Left colon op.d.4; CEA = 2.7	28 g	Mab 23 ^{131}I	1,052	38.4	11.3	(3.4)** 2.4***	7.8	(4.9) 3.8	45.4	(0.8) 3.8
		N.-IgG ^{125}I	77	2.8	2.0	(1.4)	2.1	(1.3)	13.3	(0.21)
81) Sigmoid op.d.5; CEA = 150	50 g	F(ab^1), 23 ^{131}I	897	17.9	2.4	(7.4)** 4.6***	1.8	(10.1) 6.7	7	(2.6) 8.4
		N.-IgG ^{125}I	184	3.7	2.3	(1.6)	2.4	(1.5)	12	(0.31)

* Abbreviations: Tot.nCi Tumour = Total radioactivity in tumour in nanoCuries; nCi/g = concentration in nCi per gram; N.mucosa = dissected normal mucosa; N.serosa = dissected external bowel wall; op.d.6 = patient operated 6 days after injection. CEA = 1.2 means: serum CEA level of 1.2 ng per ml (28).

** Factors of radioactivity uptake in tumor (indicated in parantheses) are obtained by dividing the concentration of radioactivity in tumor by the concentration of radioactivity either in N.mucosa, N.serosa or serum.

*** Specificity indices (underlined figures) are calculated by dividing the factor of radioactivity uptake obtained for ^{131}I antibody by the same factor obtained for ^{125}I normal IgG.

pares the tumor with the bowel wall stripped from the mucosa (normal serosa). In contrast, the same factors calculated for the control normal IgG ranged between 0.8–1.6. If one divides the factors of uptake calculated for antibodies by those calculated for normal IgG one obtains the indices of specific tumor lacalisation of antibodies. The average of these indices for the 4 patients analysed here is 4.3 (range 2.2–8.4). The 4 cases presented here all had positive scans.

Discussion

It is evident from this brief review of our recent results that the method of immunoscintigraphy for the detection of solid tumors can be improved by the use of Mabs selected for higher specificity and affinity for CEA. Other possibilities of improvement of immunoscintigraphy includes the use of Mabs against newly discovered tumor markers and the use of other isotopes for the radiolabeling of Mabs. In collaboration with the group of H. Koprowski, we have recently shown that a Mab (17-1A) directed against a new colorectal carcinoma marker can be used for the detection by scintigraphy of these tumors in patients (17). Other groups have used a Mab raised against human osteosarcoma to detect colorectal carcinomas (18) or Mabs against milk fat globule antigens to detect human carcinoma of various origin (19). It is clear that all these results need to be confirmed on well controlled clinical studies before they can be recommended for large scale use.

Concerning new isotopes, chelates, such as diethylenetriamine pentaacetic acid (DTPA) have been used to label antibodies with ^{111}Indium (20, 21) which in terms of specific energy and physical half live is particularly suitable for immunoscintigraphy. An advantage of DTPA is that it can also be used to label antibodies with different alpha-emitting isotopes which represent the best potential agents for the destruction of tumor cells (21). Critical experimental investigations are, however, necessary, before such type of radioimmunotherapy can be considered for the treatment of cancer patients.

References

Pressman, D. and Korngold, L. The in vivo localization of anti-Wagner osteogenic sarcoma antibodies. Cancer 6: 619–623, 1953.

Bale, W.F., Spar, I.L., Goodland, R.L. and Wolfe, D.E. In vivo and in vitro studies of labeled antibodies against rat kidney and Walker carcinoma. Proc. Soc. Exp. Biol. Med. 89: 564–568, 1955.

Mach, J.-P., Carrel, S., Merenda, C., Sordat, B. and Cerottini, J.-C. In vivo localisation of radiolabeled antibodies to carcinoembryonic antigen in human colon carcinoma grafted into nude mice. Nature 248: 704–706, 1974.

Goldenberg, D.M., Preston, D.F., Primus, F.J. and Hansen, H.J. Photoscan localization of GW-39 tumors in hamsters using radiolabeled anti-carcinoembryonic antigen immunoglobulin G. Cancer Res. 34: 1–9, 1974.

Hoffer, P.B., Lathrop, K., Bekerman, G., Fang, V.S. and Refetoff, S. Use of ^{131}I-CEA antibody as a tumor scanning agent. J. Nucl. Med. 15: 323–327, 1974.

Goldenberg, D.M., DeLand, F., Enishin, K., Bennett, S., Primus, F.J., van Nagell, J.R., Estes, N., DeSimone, P. and Rayburn, P. Use of radiolabeled antibodies to carcinoembryonic antigen for the detection and localization of divere cancers by external photoscanning. N. Engl. J. Med. 298: 1384–1388, 1978.

Goldenberg, D.M., Kim, E.D., DeLand, F.H., Bennett, S. and Primus, F.J. Radioimmunodetection of cancer with radioactive antibodies to carcinoembryonic antigen. Cancer Res. 40: 2984–2992, 1980.

Mach, J.-P., Carrel, S., Forni, M., Ritschard, J., Donath, A. and Alberto, P. Tumor localization of radiolabeled antibodies against carcinoembryonic antigen in patients with carcinoma. N. Engl. J. Med. 303: 5–10, 1980.

Mach, J.-P., Forni, M., Ritschard, J., Buchegger, F., Carrel, S., Widgren, S., Donath, A. and Alberto, P. Use and limitations of radiolabeled anti-CEA antibodies and their fragments for photoscanning detection of human colorectal carcinomas. Oncodevelop. Biol. Med. 1: 49–69, 1980.

Order, S.E., Klein, J.L., Ettinger, D., Alderson, P., Siegleman, S. and Leichner, P. Use of isotopic immunoglobulin in therapy. Cancer Res. 40, 3002–3007, 1980.

Mach, J.-P., Buchegger, F., Forni, M., Ritschard, J., Berche, C., Lumbroso, J.D., Schreyer, M., Girardet, Ch., Accolla, R.S. and Carrel, S. Use of radiolabelled monoclonal anti-CEA antibodies for the detection of human carcinomas by external photoscanning and tomoscintigraphy. Immunology Today 2: 239–249, 1981.

Accolla, R.S., Carell, S. and Mach, J.-P. Monoclonal antibodies specific for carcinoembryonic antigen and produced by two hybrid cell lines. Proc. Natl. Acad. Sci. (USA) 77: 563–566, 1980.

Haskell, C.M., Buchegger, F., Schreyer, M., Carrel, S. and Mach J.-P. In vitro screening of new monoclonal anti-CEA antibodies for radioimaging of human colorectal carcinomas. In R.E. Langman, I.S. Trowbridge and R. Dulbecco (Eds). Monoclonal antibodies and Cancer Academic Press New-York (in press).

Buchegger, F., Haskell, C.M., Schreyer, M., Scazziga, B.R., Randin, S., Carrel, S. and Mach J.-P. Radiolabeled fragments of monoclonal anti-CEA antibodies for localization of human colon carcinoma grafted into nude mice, submitted for publication.

Kuhl, D.E. and Edwards, R.D. Image separation radioisotope scanning. Radiology 80: 653–662, 1963.

Berche, C., Mach, J.-P., Lumbroso, J.-D., Langlais, C., Aubry, F., Buchegger, F., Carrel, S., Rougier, P., Parmentier, C. and Tubiana, M. Tomoscintigraphy for detecting gastrointestinal and medullary thyroid cancers: First clinical results using radiolabelled monoclonal antibodies against carcinoembryonic antigen. Brit. Med. J. 285: 1447–1451, 1982.

Mach, J.-P., Chatal, J.-F., Lumbroso, J.-D., Buchegger, F., Forni, M., Ritschard, J., Berche, C., Douillard, J.-Y., Carrel, S., Herlyn, M., Steplewski, Z. and Koprowski, H. Submitted for publication.

Farrands, P.A., Pimm, M.V., Embleton, M.J., Perkins, A.C., Hardy, J.D., Balwin, R.W., and Hardcastel, J.D. Radioimmunodetection of human colorectal cancers by an anti-tumor monoclonal antibody. Lancet, II: 397–400, 1982.

Epenetos, A.-A., Mather S., Granowska, M., Nimmon, C.C., Hawkins, L.R., Britton, K.E., Shepherd, J., Taylor-Papadimitriou, J., Durbin, H., Malpas, J.S. and Bodmer, W.F. Targeting of iodine-123-labelled tumour associated monoclonal antibodies to ovarian, breast and gastrointestinal tumours. Lancet II: 999–1005, 1982.

Khaw, B., Fallon, J.T., Strauss, H.W. and Haber, E. Myocardial infarct imaging of antibodies to canine cardiac myosin with Indium-111-diethylenetriamine pentaacetic acid. Science 209: 295–297, 1980.

Scheinberg, D.A., Strand, M. and Gansow, O.A. Tumor imaging with radioactive metal chelates conjugated to monoclonal antibodies. Science 215: 1511–1513, 1982.

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Contribution of epidemiology to etiological research on cancer

Bernard Junod

Zusammenfassung: Beitrag der Epidemiologie zur Erforschung der Aetiologie der malignen Tumoren

Die Epidemiologie, das heisst das Studium der Verteilung der Bestimmungsfaktoren und der Frequenz der Krankheiten beim Menschen, hat zwei wichtige Beiträge zur Erforschung der Aetiologie der malignen Tumoren geleistet. Erstens, sie hat die Wichtigkeit der Umgebungsfaktoren und dadurch die potentiellen Möglichkeiten der primären Praevention der malignen Tumoren aufgezeigt. Zweitens, sie förderte die Kenntnisse über grundlegende Mechanismen dieser Krankheit zu erwerben. Nach einem kurzen Überblick der Methode der Risikofaktorenidentifikation und nach der Erwähnung der wichtigsten Ursachen der malignen Tumoren, werden drei Aspekte in Bezug auf die Karzinogenese epidemiologisch behandelt. Die Theorie der Karzinogenese in mehreren Entwicklungsstufen; die Dosis-Wirkung-Beziehung und die Interaktion zwischen zwei Risikofaktoren. Diese drei Aspekte zeigen die Übereinstimmung der erhaltenen Resultate durch die biologische und epidemiologische Arbeitsweise. Schliesslich wird eine laufende Forschungsarbeit über die vermutete Karzinogene in gleichartigen alkoholischen Getränken als Beispiel eines möglichen Vorteils der Zusammenarbeit zwischen Biologen und Epidemiologen erwähnt.

Résumé: Contribution de l'épidémiologie à la recherche sur l'étiologie des tumeurs malignes

L'épidémiologie, c'est-à-dire l'étude de la distribution des déterminants et de la fréquence des maladies chez l'homme, a apporté deux types de contributions majeures à la recherche de l'étiologie du cancer. Premièrement, elle a fait la démonstration de l'importance des facteurs de risque environnementaux et par là du potentiel de prévention primaire du cancer. Deuxiémement, elle a favorisé l'acquisition de connaissances sur les mécanismes fondamentaux de cette maladie. Après une brève revue de la méthode d'identification des facteurs de risque et l'énoncé des principales causes du cancer, trois aspects relatifs à la carcinogénèse sont envisagés du point de vue épidémiologique: la théorie de la carcinogénèse à étapes multiples, la relation dose-effet et l'interaction entre deux facteurs. Ces trois aspects mettent en évidence la cohérence entre résultats obtenus par les approches biologique et épidémiologique. Enfin, une recherche en cours sur les carcinogenes suspectés dans les congénères des boissons alcooliques est citée comme une illustration du bénéfice potentiel d'une collaboration entre épidémiologistes et biologistes.

Introduction

Epidemiology differs from other medical sciences by the scale of its field of observation. Biology focuses more and more on molecular structures, whereas the epidemiological approach relies on the observation of populations. It is the science of disease frequency.

For an epidemiologist, the knowledge of etiology is mainly assimilated to the identification of risk factors, i.e. the variables statistically associated with the advent of a disease. An etiological factor is not always a sufficient cause as such. It is frequently a component of a sufficient cause in the complex network resulting in cancer.

Numerous risk factors have been discovered by epidemiological research (see tab. 1). In a recent textbook of cancer epidemiology, a

section deals with the most important causes of cancer (Schottenfeld and Fraumeni 1982). One chapter is specifically devoted to each determinant subject to modification and to the familial predisposition mentioned on table 1. This list illustrates the ordinary approach of cancer etiology by epidemiologists. In fact, as shown in this paper, some epidemiological results contribute to the acquisition of knowledge on fundamental mechanisms of cancer. However, the identification of the risk factors subject to modification and the demonstration of the potential of primary prevention doubtlessly represent the main contribution of epidemiology in the fight against cancer.

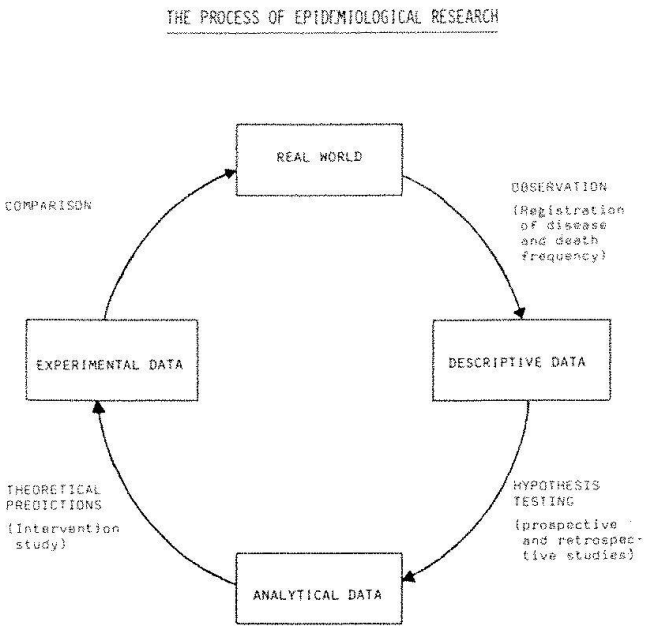

Fig. 1. The process of epidemiological research.

The epidemiological method

There are many definitions of epidemiology. Mac Mahon, in 1970, has given a concise description which fits well to the process of research in this field: "Epidemiology is the study of the distribution of disease and the search for the determinants of disease frequency in man". The successive steps of epidemiological research are illustrated on figure 1. The observation of the complex real world based on a cancer registry gives the frequency distribution of cancer. About 80 tumor registries have been selected by the International Agency against Cancer to be published in a fundamental descriptive work: "Cancer incidence in Five Continents" (Waterhouse 1982). Three of them are in operation in the French speaking part of Switzerland. The quality of their data is regularly checked by the International Agency for Research on Cancer.

The descriptive data can suggest some hypotheses concerning the determinants. This is where analytical epidemiology, as described on table 2, plays its part. If the quality of data is sufficient, a conclusion is possible by testing the statistical association between the suspected agent and cancer. Most causes have been found that way.

The effect of modification of one or more risk factors can then be introduced into a model and tested by experimental epidemiology. Basically, there are two possibilities; either one diminishes or one increases the risk in an intervention group to be compared to a reference group. Few results could be obtained by the reduction of a risk factor: numerous practical problems including ethical preoccupations preclude a wide utilisation of this method. On the other hand, catastrophes like the bombing of Hiroshima and Nagasaki or the present introduction of cigarette smoking in Africa are particularly productive from an epidemiological point of view!

Table 1. The main determinants of cancer

Subject to modification	Not subject to modification
— Tobacco	— Sex
— Alcohol	— Age
— Ionizing radiation	— Familial predisposition
— Drugs	
— Occupation	
— Air and water pollution	
— Diet	
— Viruses	
— Parasites	

Contribution of epidemiology to the comprehension of cancer mechanisms

The complementary approach of biology and epidemiology on etiological research will be illustrated here with respect to 3 fields: 1)

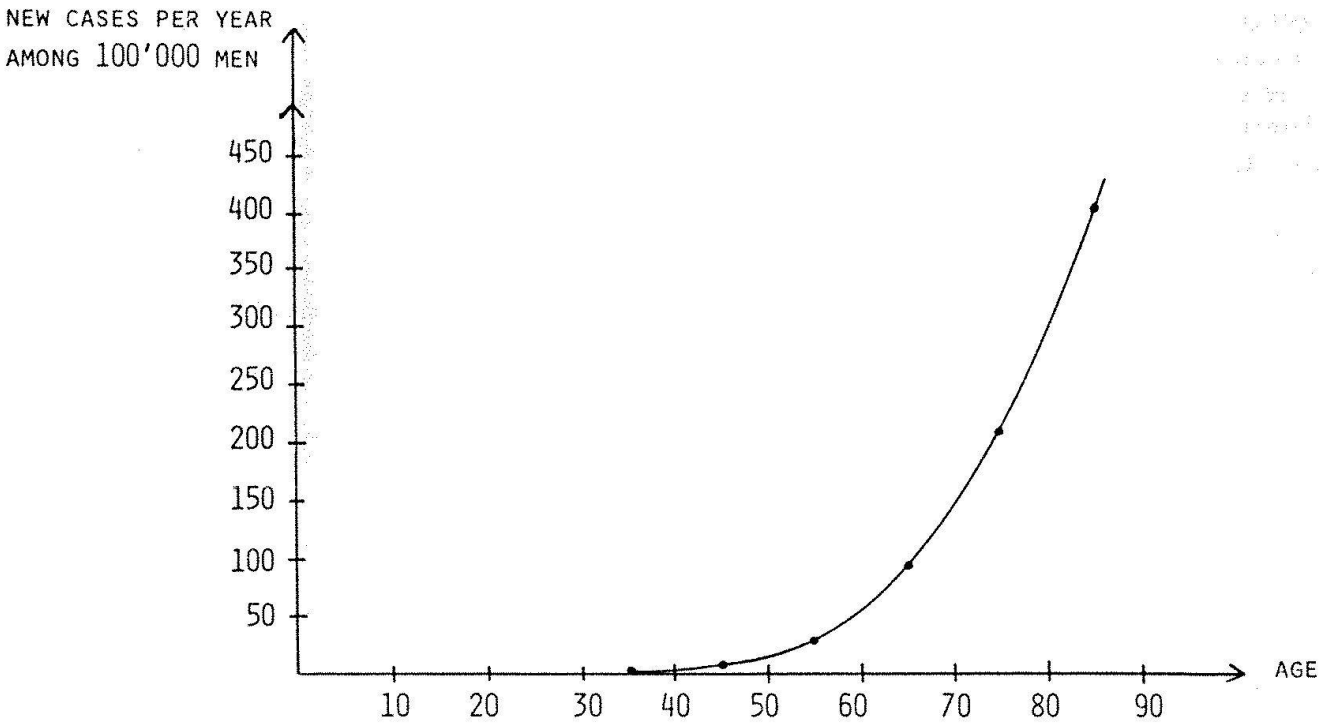

Fig. 2. Incidence of cancer of the stomach – male population – Vaud 1974–1978.

the multi-stage theory of carcinogenesis, 2) the dose-effect relation of one factor, and, 3) the interaction between two factors.

1. The multi-stage theory of carcinogenesis

This theory has been proposed by H.G. Muller (1951) and C.O. Nordling (1953). It derives from a basic epidemiological observation. The increase of cancer mortality to the 5th or 6th power of age (fig. 2) illustrates this phenomenon for stomach cancer in the Canton of Vaud. This theory presupposes that a cell originates a cancer after a certain number of transformations. Armitage and Doll (1957) have formulated the following hypothesis: each transformation occurs according to a specific transition rate at each stage. The resulting models are often too complex for a valid interpretation of the differences between prevision and observation. In a review on this subject, Peto (1977) points out the distortions introduced in such comparisons by the time trends, the inaccuracies of registration and by the multiple etiologies. Under these circumstances, the utility of this theory is somewhat questionable for cancer etiology. It is so general that it could be applied to all etiological situations. A model including fewer parameters would present more instructive features. Moolgavkar and Venzon (1979) have demonstrated that a two-stage model can explain a graph as shown on figure 2. The two-step model corresponds to the well admitted concepts of initiation and tumor promotion as defined by biologists.

Table 2. Analytical epidemiology (prospective and retrospective studies)

1. Distribution of N people according to exposure and outcome:

		Cancer (outcome) present	absent	total
Factor under investigation (exposure)	present	FC	$F\bar{C}$	F
	absent	$\bar{F}C$	$\bar{F}\bar{C}$	$\bar{F}$
	Total	C	$\bar{C}$	N

2. Definitions
Prospective study: F and $\bar{F}$ people are selected according to their exposure
Retrospective study: C and $\bar{C}$ people are selected according to the outcome variable

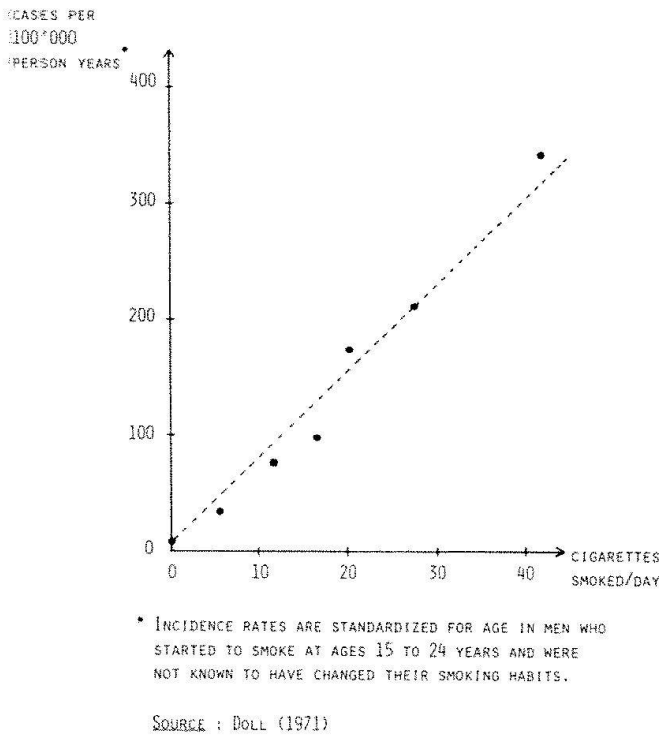

Fig. 3. Incidence rate of bronchial carcinoma versus rate of cigarette smoking.

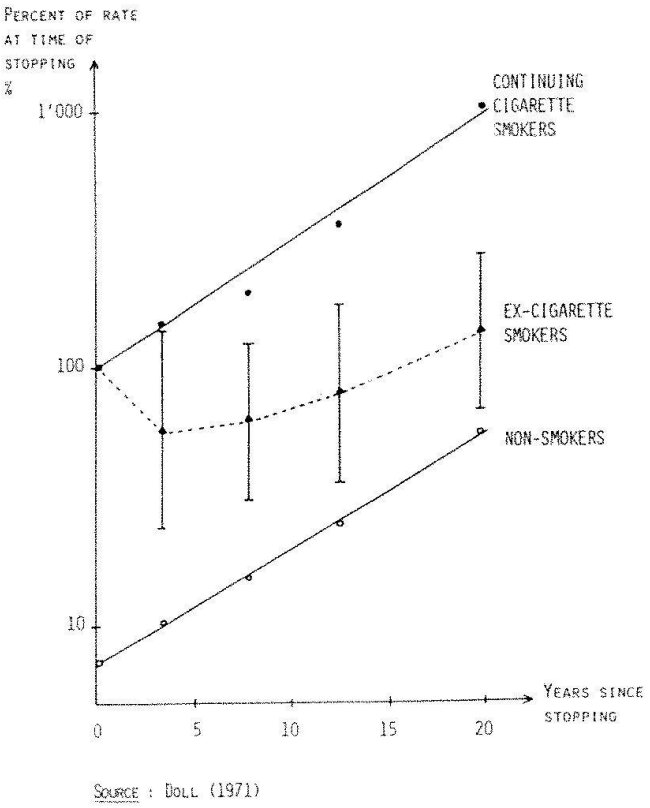

Fig. 4. Incidence rate of bronchial carcinoma versus time since cigarette smoking was stopped, compared with rates in continuing smokers and non-smokers.

2. Relationship between dose and effect

Analytical epidemiology gives a large importance to the dose-response relationship in order to interpret the causality envolved in the statistical association between an investigated factor and the disease. When a factor has been recognized as a cause, the function relating to the intensity of exposure with the frequency of the disease affords further elements suggesting possible mechanisms of cancer appearance.

The large tobacco consumption in countries where epidemiology acquired its experience and reputation is doubtlessly the main determinant of the study of bronchial carcinoma. As shown on the graph, figure 3, with a constant exposure time, the simple proportionality between tobacco consumption and the frequency of bronchial carcinoma can be observed. The same rule is observed for ionizing radiations with a reasonable dose and leukemia (Brown 1976 and Upton 1977) as well as for ultraviolets and skin cancer (Cutchis 1975). This relation is compatible with the fact that only one transition rate is affected by tobacco consumption. Moreover, if comparing cancer evolution of smokers and ex-smokers, one can suppose that tobacco consumption only interferes at the last stage of tumor development. As shown on the graph, figure 4, if the curb for ex-smokers does not increase, it does not attain the curb of non-smokers.

In the field of ionizing radiations, Land and Norman (1978) have analysed the distribution of induction periods for several cancers. They conclude by supposing that for two-stage models, radiations only affect the first stage for lung and breast cancer because of the long induction period, whereas the second stage is affected for chronic granulocytic leukemia for which the latent period is short. Furthermore, epidemiological observations have not yet revealed all new and unexpected elements; the graph, figure 5, shows the dose-effect relationship of ionizing radiations. The first part of the curb has been documented in detail by observations made on survivors of the Hiroshima and Nagasaki

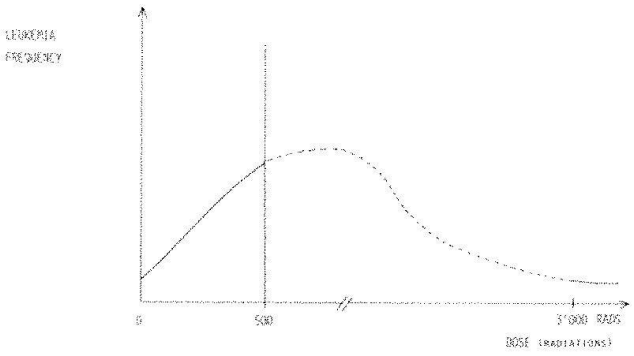

Fig. 5. Ionizing radiations and leukemia.

bombings (National Research Council 1980); in order to resist to more than 500 rads, the dose must be spread over a long period of time. It is precisely this which happens with cancer treatment. It has been proven that the rate of leukemia on strongly radiated patients does not increase. Moreover, it seems that lymphoma is more scarce on strongly radiated patients. One can thus presume that epidemiological data are an illustration of the "killing effect" described in vitro.

3. Interaction between two factors

The relative risk obtained for the combination of two factors at several exposure levels is the result of the mechanism in cancer etiology.
The data given by Rothman and Keller (1972) shown on table 3 are compatible with a multiplicative model as opposed to an additive one. This type of interaction can be observed for the combination of an exposure to asbestos and to tobacco (Saracci 1977). The multiplication of effects of two carcinogens is compatible with a multi-stage model where each one interferes at a different stage of the causal process. A proportional effect is thus obtained at each level of a factor.

Table 3. Relative risk of oral cancer according to level of exposure to smoking and alcohol.

		Smoking (Cigarette equivalent/day)		
		0	<20	40+
Alcohol (ml/day)	0	1.00	1.52	2.43
	11.8–45.8	1.60	4.36	8.21
	45.8+	2.33	4.13	15.5

Source: Rothman and Keller (1972)

Potential profits of a better cooperation between epidemiologists and biologists

The identification of substances responsible for cancer on man can largely benefit of cooperation between epidemiologists and biologists. Certain epidemiological data allow us to suppose that congeners of certain alcoholic drinks can engender cancer of the upper digestive tract. In order to test this hypothesis and to identify risk engendering drinks, the epidemiological approach is doubtlessly the right one (Junod and Pasche 1978). By questioning patients with tumors and alcoholics on the type of drinks they use, it is possible to select a limited number of wines and alcohols.
The detection of carcinogenic substances can then benefit of the biological methods used, such as the identification of mutagenic properties of these drinks on certain bacterial strains (Ames 1973). A study of this type is presently being carried out in Lausanne and will allow, within short, to determine the existence and the nature of drinks containing carcinogenes.

Conclusion

The principal result of epidemiology on cancer etiology is doubtlessly the demonstration of the importance of environmental factors in its largest meaning. Higginson and Muir (1977) have reexamined epidemiological data justifying the fact that 80% of human cancers have an environmental origin. This is not, however, a reason to minimise the importance of the acquisition of knowledge in the mechanisms of cancer, but there is no doubt that such a result could have an influence on primary cancer prevention. Even if this exceeds fundamental research it nevertheless concerns all those who fight against cancer.

References

Ames, B.N., Durston, W.E., et al. 1973: Carcinogens are mutagens. A simple test system combining liver homogenates for activation and bacteria for detection Proceedings of the National Academy of Sciences of the U.S.A., vol. 70, 1973, Nr 8, 2281–2285.

Armitage, P., Doll, R. 1957: Stochastic models for carcinogenesis in relation to the age distribution of human cancer. British Journal of Cancer, vol. 11, 1957, 161–169.

Brown, J.M. 1976: Linearity versus non linearity of dose response for radiation carcinogenesis. Health Physics, vol. 31, 1976, 231–245.

Cutchis, P. 1975: Estimates of increase in skin cancer incidence with time following a decrease in stratospheric ozone. Institute for Defense Analyses, Science and Technology Division, IDA Log No HQ 74-16869, 1975, 1–26.

Doll, R. 1971: Cancer and aging. The epidemiologic evidence. In: Clark, R.L., Cumeley, R.W., et al., eds.: Oncology 1970. Tenth International Cancer Congress, vol. 5. Yearbook Medical Publishers, Chicago, 1971, 1–28.

Higginson, J., Muir, C.S. 1977: Détermination de l'importance des facteurs environnementaux dans le cancer humain. Rôle de l'épidémiologie. Bulletin du Cancer 64, 1977, No 3, 365–384.

Junod, B., Pasche, R. 1978: Etio-épidémiologie des cancers de la bouche et du pharynx en Suisse. Schweizerische Medizinische Wochenschrift, Band 108, 1978, 882–887.

Land, C.E., Norman, J.E. 1978: The latent periods of radiogenic cancer occurring among Japanese A-bomb survivors. In: International Atomic Energy Agency Symposium on the Late Biological Effects of Ionizing Radiation. Radiation Effects Research Foundation, Hiroshima; National Academy of Sciences, Medical Follow-up Agency, Washington, D.C.

MacMahon, B., Pugh, Th.F. 1970: Epidemiology. Principles and Methods. Little, Brown and Company, Boston.

Moolgavkar, S.H., Venzon, D.J. 1979: Two-event models for carcinogenesis. Incidence curves for childhood and adult tumors. Mathematical Biosciences, vol. 47, 1979, 55–77.

Müller, H.J. 1951: Radiation damage to the genetic material. Science in Progress, vol. 7, 1951, 93–493.

National Research Council, Committee on the Biological Effects of Ionizing Radiations 1980: The effects on populations of exposure to low levels of ionozing radiation 1980. National Academy Press, Washington, D.C.

Nordling, C.O. 1953: A new theory on the cancer inducing mechanism. British Journal of Cancer, vol. 7, 1953, 68–72.

Peto, R. 1977: Epidemiology multistage models and short-term mutagenicity test. In: Hiatt, H.H., et al., eds: Origins of Human Cancer, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Rothman, K., Keller, A. 1972: The effect of joint exposure to alcohol and tobacco on risk of cancer of the mouth and pharynx. Journal of Chronic Diseases, vol. 25, 1972, 711–716.

Saracci, R. 1977: Asbestos and lung cancer. An analysis of the epidemiological evidence on the asbestos-smoking interaction. International Journal of Cancer, vol. 20, 1977, 323–331.

Schottenfeld, D., Fraumeni, J.F., Jr. 1982: Cancer Epidemiology and Prevention. W.B. Saunders, Philadelphia, London.

Upton, A.C. 1977: Radiations effects. In: Hiatt, H.H. et al., eds.: Origins of Human Cancer. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Waterhouse, J., Muir, C. et al., eds. 1982: Cancer incidence in five continents, vol. 4. IARC Scientific Publications, Nr. 42. International Agency for Research on Cancer, Lyon.

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